Price ~~Rs. 2-8-0~~ Rs 2 [*Vol. 32 A, Part II, pp. 29 to 40*

JOURNAL

OF THE

INDIAN INSTITUTE OF SCIENCE

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THE PROBLEM OF HAPLOIDY IN YEASTS

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1950

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Introduction

The recent contribution of Phaff and Mrak (1948) on "Sporulation in Yeasts" brought into broad relief the contradictory conclusions that one comes across in the published literature. We have been feeling that a clarification of these questions is imperative for any ordered advance in our knowledge.

The accidental discovery of a two chromosome brewery yeast some four years ago (Subramaniam, 1946) rendered it possible to plan a long-range programme of investigations on the Cytogenetics of Yeasts. It is surprising that many of the genetic investigations have been carried out on yeasts purchased from the market (Lindegren, 1945 *a*; Winge and Laustsen, 1937). There is an inherent disadvantage when using such strains, for, virtually nothing is known regarding their previous history or mode of origin. No organized attempt has been made, before our entry into the field, to render possible investigations on the cytology of yeasts on rational lines (Subramaniam, 1947; 1948 *a*). The genetical investigations even to-day are carried out on yeast strains whose chromosome constitutions are unknown. Is not a knowledge of the cytology of the yeast strains an essential pre-requisite for any advance in our knowledge of the genetics of yeasts? The fruitful association of cytology and genetics has been responsible for the rapid advances in our knowledge of heredity in higher organisms during the past three decades. Reasonably, therefore, one can expect that rapid advances in the genetics of yeasts would be possible only if investigations are carried out with strains of known chromosome constitution. If the cytology of yeasts is in a confused state, a clarification of the confusion should precede and not succeed genetical investigations. If the above primary condition is accepted, then, is it not legitimate to question the revolutionary claims (Lindegren, 1945 *b*; Spiegelman, 1946) advanced regarding cytoplasmic inheritance in yeasts? Investigators on higher plants and animals, generally unaware of the bristling contradictions in yeast literature, seem to accept some of the conclusions without question.

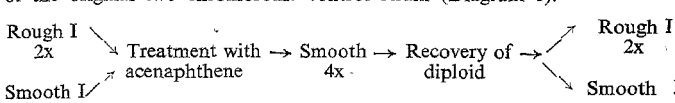
From the very commencement of investigations in this laboratory, it was realized that ordered advance would be possible only if attention was

concentrated on a single strain and its mutants. For the past four years the co-ordinated work on the two chromosome brewery yeast, BY 1, and the sixty and odd mutants obtained from it by diverse physical and chemical agencies, has been making us acutely conscious of the unsubstantiated nature of many of the fundamental assumptions. A critical analysis of some of the problems demanding clarification is attempted not in a destructive but in a constructive spirit.

Haploidy in Yeasts

A seasonal variation in the characteristics of the giant colonies of our two chromosome control strain was interpreted on the basis of the existence of multiple alleles and the selective action of the environment (Subramaniam and Krishna Murthy, 1948; Subramaniam, Ranganathan and Krishna Murthy, 1948; Mallya and Subramaniam, 1949). A duplication of the chromosome complement was possible by treatment with acenaphthene (Subramaniam, 1945; 1947). The autotetraploid obtained by treating the *Smooth I* and *Rough I* types of colonies (Subramaniam and Ranganathan, 1948; Subramaniam and Krishna Murthy, 1949) gave a smooth giant colony which was highly stable in its characteristics.

Naturally, the next problem was whether the original diploid could be recovered. Very recently, investigations planned with an entirely different end in view, rendered possible a recovery of the diploid (Duraiswami and Subramaniam, 1950), and what is more, the recovered diploid showed a seasonal variation in the characteristics of its giant colonies similar to that of the original two chromosome control strain (Diagram 1).



The autotetraploid produces spores and the giant colonies show very little variation from season to season indicating their high stability. The two chromosome control as well as the recovered diploid produce spores. But their colonies show a seasonal variability in their sculpturing and periodically show sectors. We know from the cytological evidence that the control strain is a diploid, but according to the criteria on which Lindegren (1945 *a*, *b*) distinguished "diploids" from "haploids", our tetraploid has to be classified as a diploid and our diploid as a haploid ! For, according to Lindegren diploids are highly stable, possess large cells and produce smooth colonies without sectors. The haplophase cultures on the other hand,

produce rough colonies which are often sectored. But as far back as 1937 Winge and Laustsen illustrated in their Plates VI, VII and VIII " diploid " colonies which were rough. Further, Winge (1944) illustrates in his Plate VIII a series of photographs of the " diploid " *Saccharomyces unisporus* showing various types of sectors. It would be evident that the giant colony characteristics unsubstantiated by cytological evidence, are valueless for differentiation between " haploids " and " diploids ".

Thus, a critical analysis of the criteria on which haploids are differentiated from diploids became necessary. The characteristics taken into consideration by Winge are given in Table I. It would be seen that Winge and Laustsen (1937) assume that all " haploids " arise by the direct germination of the spores. If the previous history of our autotetraploid strain was unknown, on the accepted lines of argument, it would have been classified as a diploid. It may in a general way agree with the other so-called " diploids " in its general characteristics, except for the fact that its cells are generally round. But, when a spore of the above autotetraploid strain is allowed to germinate, the " haploid " thus obtained would not satisfy the conditions on which " haploids " are identified. It possesses oval cells, shows " long shoot " (Winge and Laustsen, 1937) growth and produces spores. Except for the fact that it originates by the germination of a spore, its characters are those of the so-called " diploids ". Thus there appear to be discrepancies regarding even the criteria, between Winge and Laustsen (1937, 1940) and Lindegren (1945 *a*, *b*), for, the latter investigator considers that " haploids " can occasionally form spores (Lindegren and Lindegren, 1946, p. 128).

TABLE I

HAPLOIDS

Author	Origin	Size	Shape	Mode of budding	Spore formation	Giant colony
Winge	Spore germination	Small	Round	Proximal budding resulting in " *short shoot* " growth	Nil	?
Lindegren	do	Small	Round	?	A few sporulate	Rough; sectored
			DIPLOIDS			
Winge	Spore, cell or nuclear fusion	Large	Oval	Distal budding resulting in " long shoot " growth	Present	Rough or smooth
Lindegren	do	Large	Ellipsoidal	?	Present	Smooth not sectored

From Table I it would be found that there is agreement between these workers only as regards (1) shape, (2) size and (3) mode of aggregation of cells. Yeast cells are polymorphic and their shape and size depend on the nature of the media, quantity seeded and time of examination (Guilliermond, 1920). Haploids showing diploid cell shape, size and mode of budding are recorded by both the investigators (Winge, 1935, pp. 95 and 98; Lindegren, 1945 *b*, p. 113). Lindegren (1945 *b*) claims not only that one could classify the majority of the cultures as "haploid" or "diploid" by mere microscopic examination, but that the "terms haplophase and diplophase can be used as definitely in speaking of yeasts as of organisms in which the cytological evidence is more complete" (p. 113). From the evaluation of the criteria given above, only one conclusion is possible. Identifications have to be based on cytological data and not merely on morphological ones. Further, the use of the terms "haploid" and "haplophase" as if they are synonymous, has to be seriously deprecated. From what we know of higher plants, they are not. A haplophase is a gametophyte, while a haploid is a sporophyte. One is surprised to find that it is on such quick sands that huge edifices (Lindegren, 1945 *b*; Lindegren and Lindegren, 1946; Spiegelman, 1946) have been built up.

Various Types of Haploids

Both Winge and Lausten and Lindegren offer evidence for the possibility of hybridization. We have adduced evidence that polyploidy could be induced in yeast by diverse agencies. The chromosome numbers recorded by various investigators for yeasts indicate degrees of polyploidy if we consider the basic number to be two (Table II).

Table II

2 Chromosomes	4 Chromosomes	8 Chromosomes
Badian (1938) ..	Fuhrmann (1906)	Kater (1927)
Subramaniam (1946) ..	Swellengrebel (1905) Sinoto and Yuasa (1941) Subramaniam (1947) Ranganathan and Subramaniam (1948)	Renaud (1938)

Once the possibility of auto- and allopolyploidy is conceded, there are chances of confusing "real haploids" with "polyhaploids" (Darlington, 1937, p. 580). Lacking cytological data and a knowledge of the previous history of the strains, the investigators could not have differentiated between these various types, for, even among "polyhaploids" there ought to be

different types. A variety of such *reduced* forms were described by Satava (quoted by Winge and Laustsen, 1937) even before Winge or Lindegren. The *Type I* of Satava formed spores either in small numbers or with reduced budding vigour, while the *Types II* and *III* were incapable of sporulation. The *Types I* and *II* were capable of regulating their cell size unlike *Type III* which lacked such an ability with the result that cells in such colonies were irregular, longish and amœboid.

The scepticism regarding Satava's observations appears to be due to the belief expressed by him that *reduced* forms can arise not only by the direct germination of spores but also through inanition and that *reduced* forms could regain their normal condition when cultured in normal media. Winge and Laustsen (1937) admit that their discoveries only confirm several of Satava's observations. Those observations about which Winge was sceptical were confirmed independently by Fabian and McCullough (1934) in their study on " Dissociation in Yeasts ". Just as Winge and Laustsen (1937) were unaware of the implications of Fabian and McCullough's work, the latter authors were not aware of Satava's observations.

Under particular environmental conditions Fabian and McCullough observed normal cells giving rise to the small *gonidial* forms. These gonidial forms reverted to the normal condition when repeatedly transferred through malt extract broth. Can we not consider these observations of Fabian and McCullough as an impartial confirmation of Satava's suggestion that not only could *reduced* forms arise through inanition, but that these *reduced* forms could be transformed into the normal type when provided with adequate nutrition ?

But that is not all. There is further confirmation of some other observations of Satava. He considered that *reduced* forms could arise as a number of small buds from a normal cell (Winge and Laustsen, 1937, pp. 109-10). Fabian and McCullough (1934) found that transformation of the normal *Smooth* form into the small *gonidial* type may be gradual or sudden. During the sudden transformation, the normal cells become refractile and their mode of budding was peculiar. " A multitude of minute buds appeared on the periphery of the cell. These minute buds upon becoming detached from the cell corresponded to the G form of the yeast " (p. 610). The remarkable similarity of the phenomenon should convince even the sceptic of its reality. Cytologically also, such a reduction is not improbable (Ranganathan and Subramaniam, 1948). Polyploid nuclei have been known to divide by multipolar mitoses with the resultant production of cells having the diploid chromosome number. Darlington (1937) considers that Winkler's observa-

tions of a halving of the chromosome number in the tetraploid *Solanum nigrum* could be explained on the above basis.

The independent confirmation of Satava's unusual observations emphasize and confirm the belief that different types of "haploids" do occur in yeasts.

Is A Haplophase Essential?

Winge and Laustsen's observations (1939 *b*) on *Saccharomycodes ludwigii* indicate that a haplophase is not essential. In *Schizosaccharomyces octosporus* (Spiegelman and Lindegren, 1945) cell fusions very early in single spore cultures effectively suppress the haplophase stage. The spores developed by the strains having varying genic and chromosomal constitutions may or may not have balanced chromosome complements. The real haploid may be unstable and as in the case of Frog's eggs induced to develop parthenogenetically (Sharp, 1934), may become diploid by somatic doubling. The spore of an autotetraploid would be capable of normal germination while that of an autohexaploid may be incapable of doing so. In the case of hybrids, the diploid may be sterile, while an allotetraploid may be fertile. Not all allopolyploids need produce spores capable of giving rise to an indefinite number of vegetative cells by parthenogenetic development. Thus, spores could be classified (Winge and Laustsen, 1940, p. 19) into two broad groups: (1) those possessing unlimited powers of proliferation and (2) those which possess very limited powers of germination. The development of a haplophase cannot, therefore, be universal.

Origin of Mating Types

The possibility of the occurrence of these two categories of spores in different strains leads to a consideration of the origin of the various mating types. The real haploid should be capable of isogamous fusion. In autotetraploids a mechanism ensuring fertilisation becomes a necessity since the spores possess a balanced diploid chromosome constitution. Our autotetraploid brewery yeast BY 3, which has been under constant observation for the past four years is highly stable and has never shown any diploid sectors. This indicates that a gene mutation should have occurred before a doubling of the chromosomes. This suggestion is reminiscent of the existence of *mating type* alleles demonstrated by Lindegren (1945 *b*) in some strains. But there is this fundamental difference between our views. *The mating types occur in autotetraploids and not in diploids.* There is considerable justification for such a view even in the work of Lindegren and Lindegren (1944). They found that the two complementary types of cells originating by the germination of ascospores which showed abundant

copulation immediately after isolation, failed to do so when kept in the vegetative condition for an year. A simple reverse mutation may explain this disappearance of the *mating type* reaction. If we consider that their starting type was an autotetraploid, then, the spores are diploid and capable of unlimited proliferation. The *mating type* alleles which are a necessity in the autotetraploid become superfluous when kept in the vegetative condition and a reverse mutation is a normal corollary.

But the occurrence of such *mating type* alleles cannot be universal. In those strains where the spores have an unbalanced genic constitution, fusion of complementary spores becomes a necessity. This would naturally become accentuated in the case of hybrid polyploids.

Mode of Origin of Torulæ

Carrying this analysis a step further, asporogenous "Torula" like forms obtained by spore germination are not sterile haploids as suggested by Winge and Laustsen and by Lindegren, but may in all probability be diploid hybrids which are sterile. Winge and Laustsen (1937) mixed two such "Torula" like strains hoping that a fusion would take place resulting in the formation of a cell capable of producing spores. It is not surprising that their attempts resulted in a failure. Vegetative cells of sterile diploid hybrids need not necessarily fuse. But if on the other hand a doubling of the chromosomes could be induced artificially, it might be possible to obtain a strain producing spores. Such phenomena are known to exist in higher plants.

The case of *Torulopsis pulcherrima* used by Punkari and Henrici (1933; 1935) is worth considering in this connection. It was asporogenous. The first description of copulation and spore formation was in a culture of *Torulopsis pulcherrima* in which a Penicillium occurred as a contaminant (Windisch, 1938, 1940). It would be interesting if proof could be obtained that under certain conditions, the metabolic products of Penicillia could induce polyploidy. The above sets of observations when viewed in the light of the theoretical possibility of obtaining sporogenous strains from asporogenous ones, should indicate that the broad classification of yeasts into those which are capable or incapable of forming spores is arbitrary and artificial.

There is a very suggestive observation of Winge and Laustsen (1937) worth considering in this connection. A spore isolated from the Danish Baking Yeast resisted their energetic efforts to convert it into a "diploid" and so they took it to be a sterile segregation product, comparable to asporo-

genous Torulæ. " That this type, nevertheless. is capable of diploidization was learned láter on, as a few colonies of diploid cells were observed in the culture flasks more than half a year later. These colonies were encountered in flasks with wort to which a two-month-old culture was transferred. In keeping with this finding, a few spore containing asci were observed when the culture was transferred on plaster block " (p. 111). Tetraploid sectors have been observed by us to occur repeatedly in diploid colonies and the above observations of Winge and Laustsen are reminiscent of the different strains of *Torulopsis pulcherrima* investigated by Punkari and Henrici (1933, 1935), Windisch (1938, 1940) and Roberts (1946).

Are we entitled to evaluate these observations in the light of our knowledge of the cytological phenomena in higher plants ? Are the " Torulæ " haploid or diploid ? The monoploid *Crepis* (Sharp, 1934) is sterile and it often produces fertile diploid branches. But meiosis even in these diploid branches is stated to be not very regular. On the other hand, a sterile diploid hybrid may give rise to a tetraploid either by the accidental fusion of unreduced gametes or by somatic doubling (Darlington, 1937, p. 188). Which of these explanations is applicable to the origin of Torulæ ? It has to be emphasized that the majority of the angiosperms are polyploids, that only rarely haploid plants have been obtained, that haploids cannot be produced to order and that most of the haploids have a tendency to become diploid by somatic doubling. When the above facts are viewed in the light of the conclusions: (1) that hybridisation is possible in yeasts, (2) that polyploidy is more common than imagined and (3) that identification of " haploids " in yeasts is based on characters considered highly variable by most investigators, the probability appears to be that Torulæ instead of being haploids may really be sterile diploid hybrids.

The above critical analysis reveals the unsatisfactory nature of some of the fundamental assumptions. The moral is obvious. Only planned cytogenetical investigations could clarify this confusion.

Summary

1. Many of the fundamental assumptions of investigators on yeast genetics are unsubstantiated. There is disagreement between the workers regarding criteria for distinguishing haploids from diploids. Haploids are identified on highly variable characters like shape, size and mode of aggregation of cells. From a critical evaluation of the criteria the necessity for identifications based on cytological data is emphasized.

2. The possibility of the occurrence of real haploids and polyhaploids is indicated. There appear to be remarkable similarities between the observations of Satava on the mode of origin of *reduced* forms and that of Fabian and McCullough on "dissociation" resulting in the production of gonidial forms.

3. The probable mode of origin of mating type alleles as a single gene mutation before duplication of the chromosome complement of a diploid is discussed and it is suggested that Torulæ may really be sterile diploid hybrids.

4. A clarification of the contradictory conclusions that one comes across in the published literature on yeasts is considered imperative for any ordered advance in our knowledge.

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Note Added in Proof.—A senior worker in the field distorting and misinterpreting the observations of others is a rare event. Yet, that is what Winge has done in a recent publication (*C. R. Lab. Carlsberg*, 1951, **25**, 85). Subramaniam and Ranganathan (*Nature*, 1946, **157**, 50) raised the question whether the peculiar behaviour of the four spores from the same ascus of the press yeast studied by Winge and Laustsen (*C. R. Lab. Carlsberg*, 1937, **22**, 99) may not be the result of an unbalanced chromosome constitution. This query was necessitated by the reported behaviour of the four spores isolated from an ascus (pp. 110–11). Spore I is said to have germinated and given rise to haploid cells. They are claimed to have become diploids later. Spores II and III, on the other hand, germinated directly into diploid cells. Curiously enough, the progeny of Spore IV, which are claimed to be haploid, "resisted" all attempts to transform them into diploid cells.

It should be remembered that the criteria for the identification of "haploids" and "diploids" employed by Winge and Laustsen are of questionable validity. Lacking cytological data, one would have expected them to have based their identification on more unequivocal criteria. They describe on the contrary, "haploids" showing diploid cell size, shape and mode of budding (1935, pp. 95, 97). What may have been a more reliable criterion, *viz.*, the ability to form spores, also proves to be of doubtful value, since they (1937) describe a "diploid" (Photo 20) incapable of sporulation. Further, we have been rather sceptical of their evidence for direct "diploidization" (Duraiswami and Subramaniam, *Cellule*, 1950, **53**, 215). In fact a perusal of their paper (1937) would show that Winge himself is not quite sure of his grounds. A relevant quotation in this connection may not be out of place. "It is, therefore, largely by indirect means that we have had to establish the fact that the single spores which germinate with elongated cells form diploid colonies, while those that germinate with round cells form haploid colonies" (p. 105).

To us these contradictory observations based on dubious criteria were objectionable and it was this which was given expression to by Subramaniam

and Ranganathan (1946). A perusal of that statement would show clearly that no reference was made to genetic segregations at all. The important fact emphasized by us is glossed over by Winge (1951, p. 92) and it is made to appear as if we are surprised that a "heterozygotic organism is capable of segregating out 4 different types in one tetrad". This glaring misinterpretation is followed by the comment that we are strangers to Genetics. The question of a heterozygote being implicated was never raised at all, since in the first instance we have not been in a position to accept their claim that haploids could be differentiated from diploids on pure morphology. When four spores from the same ascus germinate in different ways into the so-called "short-shoot" and "long-shoot" growths, and when some "haploids" resist diploidization and some of the "diploids" are unable to sporulate, the evidence from such material cannot *a priori* be taken as indicating purely Mendelian inheritance. When Winge comments on the knowledge of Genetics of others it would have been reasonable to expect his evidences to leave no lacunæ.

406-51 Printed at The Bangalore Press, Mysore Road, Bangalore City, by C. Srinivasa Rao, Superintendent.