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HAPLOIDY AND THE SPECIES CONCEPT IN YEASTS

BY

M. K. SUBRAMANIAM, M.A., D.Sc.

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Prof. M. S. THACKER
B.Sc. (Engg.) (Bri'l), M.I.E.E., F.Am.I.E.E., M.I.E. (Ind.), M.Inst.F., M.I.R.E., F.A.Sc., M.I.I.M.
Chairman of Editorial Board

HAPLOIDY AND THE SPECIES CONCEPT IN YEASTS

By M. K. SUBRAMANIAM, M.A., D.Sc.
(Cytogenetics Laboratory, Indian Institute of Science, Bangalore-3)

CONTENTS

INTRODUCTION

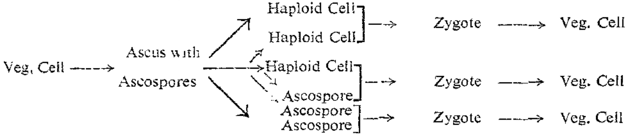
The problem of haploidy in yeasts was discussed in a previous contribution (Subramaniam, 1950) and it was indicated that there is a real possibility of the earlier investigators on yeast genetics having confused "haploids" with "polyhaploids" since identifications of "haploids" were based not on cytological data but on highly variable characters like size, shape and mode of budding of cells. It was suggested that asporogenous *Torula*-like forms may in all probability be sterile diploid hybrids and not "haploids" as suggested by Winge and Laustsen (1939) and Lindegren (1945). The case of *Torulopsis pulcherrima* was discussed in the light of the theoretical possibility that sterile diploid hybrids may give rise to fertile allotetraploids.

Hansen (Jorgensen, 1925) isolated from *S. ludwigii* (*a*) vigorously sporogenic, (*b*) mildly sporogenic and (*c*) asporogenous strains. The asporogenous strain gave rise to the sporogenous variety on repeated transfers through wort. He could transform sporogenous strains of *Saccharomyces* into asporogenous ones by culturing them at temperatures above the maximum for spore formation. "The transformation is accomplished slowly and by successive culturing; the number of sporogenic cells gradually diminish until they totally disappear. Thus may be obtained asporogenic varieties which may be maintained indefinitely. Hansen has been able to keep them for sixteen years without taking up the sporogenic property again" (Guilliermond, 1920, p. 182). Fabian and McCullough (1934) could also obtain asporogenous *gonidial* forms from sporogenous ones under specific environmental conditions. They record reversion of the small *gonidial* to the normal *smooth* condition when repeatedly transferred through malt extract broth.

Can we consider the asporogenous strains isolated as belonging to the various species of *Saccharomyces* from which they were obtained ? Apparently not ! Guilliermond (1920) lists "Asporogenic Species of Zygosaccharomyces" (p. 219) but this is a contradiction in terms. In the definition of the family *Saccharomycetes* given by him (p. 193), the important consideration taken into account is the ability to form spores. The genus *Zygosaccharomyces* is said to be characterised by copulation of vegetative cells before ascospore formation (p. 206). Under the circumstances how can one speak of "Asporogenic Zygosaccharomyces" ? As emphasized in a previous contribution (Subramaniam, 1950), the broad classification of yeasts into those which are capable or incapable of forming spores is arbitrary and artificial.

LIFE-CYCLE OF SACCHAROMYCES AND ZYGOSACCHAROMYCES

When the broad classification into asporogenous and sporogenous genera itself appears to be arbitrary, the validity of the separation of haplontic from diplontic yeasts could just be imagined. Let us first start with the life-cycle of the genus *Saccharomyces* diagrammatically presented by Phaff and Mrak (1948).



When a vegetative cell forms ascospores *meiosis is assumed to occur.* There is no definite proof that it does occur. The ascospores are thus conceived to have a "haploid" chromosome complement. The ascospore or the cells resulting from their germination fuse and the vegetative "diploid" condition is brought into being. Forgetting for the moment the existence of polyploidy in yeasts and accepting the above suggestions, an analysis becomes interesting. When conditions become unfavourable (i) there is a tendency for sporulation, (ii) meiosis precedes sporulation and (iii) spores or "haploid" cells have a tendency to fuse in pairs. Can we conclude that the tendency for fusion is the result of the possession of a monoploid chromosome complement ? *If that is so why is it that in "haplontic" yeasts vegetative cells show a tendency for fusion only at particular periods* ? From the theoretical considerations presented in connection with the probable mode of origin of mating types in yeasts (Subramaniam, 1950) it would have become apparent that these mechanisms are developed to ensure fusion of cells and thus stability as well as fertility.

If we apply the accepted assumptions on which the life-cycle of the genus *Saccharomyces* is explained to *Zygosaccharomyces* insuperable difficulties are encountered. We have to accept without any valid evidence (1) that the vegetative cells are " haploid ", (2) that under particular conditions they have a tendency to fuse, (3) that there is an immediate reduction division, (4) that the spores have only the " haploid " chromosome complement and (5) that these germinate directly. When it is realized on how many unsubstantiated assumptions identification of " haplontic " yeasts are based, it is surprising that no previous investigator ever voiced his misgivings. It appears as if constant repetitions have given the assumptions a validity which they do not possess. Thus we find Phaff and Mrak (1948) asserting: " When ascospore formation immediately follows conjugation, as is the case in most cultures of *Zygosaccharomyces*, nuclear fusion followed by reduction division *must* take place in the original zygote " (p. 265). *There is no cytological evidence that it does take place.*

MEIOSIS AND SPORULATION

With the existing data we have to evaluate the relative importance of (i) fusion and (ii) spore formation in judging the probable time of occurrence of meiosis. When we remember that " durable " cells could be formed without any reduction division and that the number of spores is variable, mere spore formation does not justify the conclusion that it must be preceded by meiosis. The necessity for a fusion of the cells requires on the other hand, more careful consideration. " Haploid " cells have a tendency to become diploid. *The sudden tendency for fusion observed in the vegetative cells of the genera Schizosaccharomyces and Zygosaccharomyces during particular stages should be the result of a reduction division in the vegetative condition.* This simple assumption would enable a rational explanation of the various types of life-cycles observed in yeasts. It is interesting to remember in this connection that the nuclear reorganization in Paramœcia described as endomixis by earlier workers turned out to be autogamy preceded by meiosis (Sonneborn, 1947). There is considerable justification for the probable occurrence of meiosis in vegetative cells. The classification of yeasts into haplontic and diplontic groups appears to be patterned on the conditions occurring in Algæ. Twelve years ago I was interested in Sporozoa (Subramaniam and Ganapati, 1938). Since similar mechanisms could develop in widely separated groups a comparison is justifiable. Encystment in Protozoa and sporulation in yeasts are mechanisms to tide over unfavourable environmental conditions. In yeasts and sporozoa, this phenomenon has become associated with a sexual process. " In the case of the gregarines two individuals become enclosed in a cyst (gametocyst), within which each gives rise to a number of gametes. The gametes unite in pairs, and the zygote thus produced itself becomes encysted in the *oocyst*, within which it divides into a number of sporozoites. In the case of the Coccidia, the zygote is encysted in the oocyst which is formed either before or after syngamy has taken place. Within the oocyst the zygote divides into a number of sporoblasts, which in their turn become encysted in sporocysts. Inside the sporocysts, the sporoblasts divide into sporozoites " (Wenyon, 1926, p. 48).

Is not one entitled to arrive at the conclusion that a reduction division need not always precede spore formation ? Even in yeasts " durable " cells are formed without any reduction division. Spore formation in the genus *Saccharomyces* appears to be preceded by meiosis since the spores usually fuse in order to give rise to the vegetative generation.

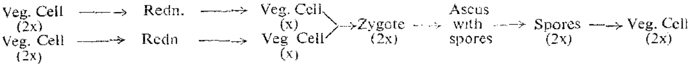
There is evidence in the published literature on yeasts that spore formation need not always be preceded by a reduction division. In *Schizosaccharomyces pombe* and *Sch. mellacei* Guilliermond (1920, p. 19) describes what he considers to be parthenogenesis. Two cells unite, but the wall separating them does not break down. The nuclei of the two cells do not fuse and each cell transforms itself into an ascus and produces spores. A rational explanation of the formation of spores in such cases becomes impossible if we proceed on the accepted basis that (*a*) the cells which fuse are " haploid " and (*b*) that a reduction division precedes spore formation, since the spores in such cases can have only sub-haploid number of chromosomes !

A NEW INTERPRETATION

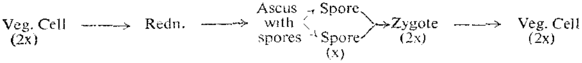
All these difficulties could be obviated if we consider that (1) the so-called " haplontic " yeasts are really diploid, (2) that unlike in the genus *Saccharomyces*, reduction division occurs in the vegetative stage, (3) that it is cells with gametic chromosome numbers that fuse and (4) that the spores have the diploid instead of the " haploid " chromosome number.

The fundamental difference between the accepted classification and the one presented above merely depends on the time when meiosis occurs and the obvious modifications necessary when such an assumption is made. The diagram of the life-cycle of *Saccharomyces* requires only minor modifications to represent that of *Schizo-* and *Zygosaccharomyces*. The difference is merely in the time of meiosis. That mutations or the environment may alter the time of reduction division is suggested by the observations of Winge and Laustsen (1939). They were able to hybridize *Zygosaccharomyces priorianus* with *Saccharomyces cerevisiæ* Rasse II. The strain of *Z. priorianus* obtained by them from Delft was not typical. " A study of the yeast in question disclosed that the spores germinated chiefly in the same manner as the *Saccharomyces* species, *i.e.*, now by spore copulation, now by haploid germination, followed sooner or later by formation of twin zygotes and monozygotes " (Winge and Laustsen, 1939, p. 340). Phaff and Mrak (1948) state: " It was also found that if this yeast were kept in culture on malt agar it showed a tendency to change from the haplophase into the diplophase but not in the opposite direction " (p. 266). A mere shift in the timing of the reduction division could convert a *Zygosaccharomyces* into a *Saccharomyces* type.

Zygosaccharomyces Type



Saccharomyces Type



Even among the different species of *Schizosaccharomyces* there is such a shift. Spiegelman and Lindegren (1945) remark that while *Sch. pombe* " can without any difficulty exist in the haplophase ", in *Sch. octosporus* the cells resulting from the direct germination of the spores " fuse rapidly to produce diploid cells " (p. 258).

There is an obvious difficulty in accepting the conclusion of Phaff and Mrak (1948) presented in their diagram said to illustrate " the manner in which *Zygosaccharomyces* (hybrid vegetative phase) may shift to the *Saccharomyces* (diploid vegetative phase) and *vice versa* " (p. 266). If according to the typical accepted convention, the *Zygosaccharomyces* vegetative cells are haploid, then these vegetative cells in the so-called *Saccharomyces* phase cannot give rise to normal " haploid " spores by a reduction division. A doubling of the chromosomes should have occurred prior to the assumption of the *Saccharomyces* phase. Further, even according to their diagram there should be a *shift* in the time of meiosis. While in the *Zygosaccharomyces* phase, meiosis succeeds zygote formation, in the *Saccharomyces* phase zygote formation succeeds meiosis. In order to explain this shift in the conventional lines a number of simultaneous changes have to be postulated.

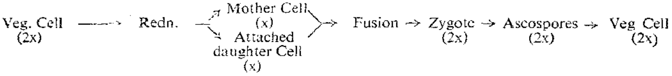
When we remember their statement that in " *Z. mandchuricus* the ratio between asci forming with and without copulation immediately prior to sporulation depended to a great extent on the substrate in which it was grown " (Phaff and Mrak, 1948, p. 266), the difficulties of a rational explanation become magnified. We have to believe, without any experimental evidence that a series of chromosomal and gene mutations can occur simultaneously.

ARE LIFE-CYCLE CHANGES GENE DETERMINED ?

According to the new interpretation, *Zygosaccharomyces* and *Saccharomyces* both of which may be assumed to be diploids *differ only in the timing of meiosis.* This may after all turn out to be governed by a single gene (*cf.* Gene determined meiotic abnormalities. Dobzhansky, 1947, p. 294; White, 1945, pp. 190–92). That this gene may mutate in either direction is suggested by some observations of Roberts (1946) which lack critical value. According to Windisch (1938, 1940) it is cells arising from germinated spores that fuse to give rise to the vegetative phase in *Torulopsis pulcherrima.* Roberts on the other hand observes: " A few indications of copulation of vegetative cells were noted. One non-pigmented colony was found to consist of cells which appeared to be copulating with the formation of conjugation tubes (Pl. II, Fig. 7) " (p. 364). While Phaff and Mrak (1948) consider that *Zygosaccharomyces* phase can change to the *Saccharomyces* type, Roberts' results indicate a shift from the *Saccharomyces* to the *Zygosaccharomyces* type.

$$\text{Zygosaccharomyces} \xrightarrow[\text{Mrak}]{\text{Phaff \&}} \text{Saccharomyces} \xrightarrow{\text{Roberts}} \text{Zygosaccharomyces}$$

Acceptance of the suggestion that (1) all yeasts are diploid or polyploid in the vegetative phase and (2) that they differ only regarding the time when reduction division occurs implies as a corollary that in the so-called haplontic yeasts meiosis occurs in the vegetative cells. If this proposition is accepted, it logically follows that such a reduction division may give rise to iso- or anisogametes. This is apparently what happens in *Debaryomyces* and *Nadsonia* (Types 3 and 5 of Phaff and Mrak, 1948).



This is only a modification of the *Zygosaccharomyces* life-cycle in that the gametes differ in size. The above scheme would enable not only a logical explanation for the occurrence of iso- and anisogamous copulations of vegetative cells but would also explain the usual occurrence of a single spore in *Nadsonia.* A reduction division may produce iso- or heterogametes and since the spores are " diploid ", the number could be variable.

Zygosaccharomyces Type (Meiosis in Vegetative Cells) ↗ Isogamous ——→ Schizosaccharomyces
Zygosaccharomyces Type (Meiosis in Vegetative Cells) ↘ Heterogamous ——→ Debaryomyces, Nadsonia.

NEED FOR REVISION

One has to remember the pregnant statement of Goldschmidt (1948): "In such well studied organisms as *Drosophila* in which numerous visible and incidentally small invisible mutants have been recombined, never has even the first step in the direction of a new species been accomplished, not to mention higher categories" (p. 470). Lodder's (1947) transformation of *Z. marxianus* into *S. macedoniensis* and *vice versa* should give one an idea of the validity of the species in yeasts !

As in bacteria (Luria, 1947) species in yeasts remain that "nebulous something which everybody calls a species but which nobody can define, describe or recognize in a fashion which is quite acceptable to the next student in the field" (Kinsey. 1942). Henrici (1941) remarks: "A number of the genera of the asporogenous yeasts find their counterpart in the sporogenous group." The cytogenetics of yeasts is yet in its infancy but enough is known about haploidy, diploidy and polyploidy to make one wonder whether a revision of the criteria employed in the broad classification into groups would not be desirable ?

SUMMARY

1. The broad classification of yeasts into haplontic and diplontic is shown to be arbitrary and artificial.

2. The tendency for fusion exhibited by the spores or haploid cells of *Saccharomyces* if assumed to be the result of the possession of a monoploid chromosome complement raises the question as to why in haplontic yeasts the vegetative cells show a tendency for fusion only at particular periods.

3. The accepted explanation of the life-cycle of haplontic yeasts appears to be based on a series of unsubstantiated assumptions. A rational explanation is possible if we assume that even the so-called haplontic yeasts are diploid in the vegetative condition, and that the tendency for fusion observed in the vegetative cells of the genera *Schizosaccharomyces* and *Zygosaccharomyces* during particular stages should be the result of a reduction division in the vegetative condition. The shift from the *Zygosaccharomyces* to the *Saccharomyces* phase could easily be explained as the result of a simple gene mutation. Derivation of the life-cycles of *Debaryomyces* and *Nadsonia* is then a simple matter since a reduction division may give rise to iso- or heterogametes.

4. The concept of species is much more nebulous in yeasts than in higher organisms and the need for a thorough revision of the criteria used even for broad classification into groups is emphasized.

REFERENCES

1. Dobzhansky, Th. (1947) .. *Genetics and Origin of Species*, Columbia Univ. Press.
2. Fabian, F. B. and N. B, McCullough (1934) "Dissociation in Yeasts," *J. Bact.*, **27**, 583–620.
3. Goldschmidt, R. B. (1948) .. "Ecotype, Ecospecies and Macroevolution," *Experientia*, **4**, 465–72
4. Guilliermond, A. (1920) .. *The Yeasts*, New York.
5. Henrici, A. T. (1941) .. "The Yeasts—Genetics, Cytology, Variation, Classification and Identification," *Bact. Revs.*, **5**, 97–179.
6. Jorgensen, A. (1925) .. *Micro-organisms and Fermentation*, London.
7. Kinsey, A. C. (1942) .. "Isolating Mechanisms in Gall Wasps," *Biol. Symp.*, **6**, 251–69.
8. Lindegren, C. C. (1945) . "Yeast Genetics. Life Cycles, Cytology, Hybridisation, Vitamin Synthesis and Adaptive Enzymes," *Bact. Revs.*, **1**, 111–70.
9. Lodder, J (1947) .. Quoted by Phaff & Mrak.
10. Luria, S. E. (1947) .. "Recent Advances in Bacterial Genetics," *Bact. Revs.*, **11**, 1–40.
11. Phaff, H. J. and E. M Mrak (1948) "Sporulation in Yeasts I," *Wallerstein Lab. Commns.*, **9**, 261–81.
12. Roberts, C. (1946) .. "A comparative Study of *Torulopsis pulcherrima* and *Taphrina deformans* in Culture," *Farlowia*, **2**, 345–83.
13. Spiegelman, S. and C. C. Lindegren (1945) "The Relation of Sporulation and Range of Variation of the Haplophase to Populational Adaptation," *J. Bact.*, **49**, 257–69.
14. Sonneborn, T. M. (1947) .. "Recent Advances in the Genetics of Paramecium and Euplotes," *Adv. in Gen.*, **1**, 264–358.
15. Subramaniam, M. K (1950) "The Problem of Haploidy in Yeasts," *J. Ind. Inst. Sci.* (In the press).
16. ——— and P. N. Ganapati (1938) "Studies on the Structure of the Golgi Apparatus I," *Cytologia*, **9**, 1–16.
17. Wenyon C. M. (1926) .. *Protozoology*, **1**, London.
18. Windisch, S. (1938) .. "Zur Kenntniss der Ascosporenbildung bei *Torulopsis pulcherrima* (Lindner) Saccardo," *Arch. Mikrobiol*, **9**, 551–54.
19. ———— (1940) .. "Entwicklungsgesichtliche Untersuchungen an *Torulopsis pulcherrima* (Lindner) Saccardo und *Candida tropicalis* (Castellani) Berkhout. Ein Beitrage zur systematik der Garungsmonilien," *ibid.*, **11**, 368–90.
20. Winge, O. and O. Laustsen (1937) "On Two Types of Spore Germination and on Genetic Segregation in *Saccharomyces* demonstrated through single spore cultures," *C. R. Lab. Carlsberg. Ser., Physiol.*, **22**, 99–119.

21. Winge, O. and O Laustsen (1939) .. "On 14 Yeast Types produced by Hybridisation," *Ibid.* **22**, 337–55.

22. White, M. J. D. (1945) .. *Animal Cytology and Evolution*, Camb. Univ. Press.

Note added in proof.—From an analysis of some fundamental problems in yeast cytogenetics, Duraiswami and Subramaniam (*Cellule*, **53**, 1950, 215) suggested some possibilities for the consideration of workers in the field. These were not given as conclusions but as possible alternatives to be kept in mind when results capable of more than one interpretation are obtained. Curiously enough these suggestions have been misinterpreted and misconstrued (Winge, *C. R. Lab. Carlsberg*, **25**, 1951, 85) to show that they are fantastic. We suggested that as a result of " meiotic aberrations " diploid spores can occur. Further elaboration of the above statement was considered unnecessary since Darlington (*Recent Advances in Cytology*, Churchill, London, 1932) considers this method (p. 64) as a possible source of polyploidy in plants. Winge comments: " In general, Duraiswami and Subramaniam attempt to explain genetic segregation and mutation in yeasts by the occurrence of chromosome disturbances " (p. 92). In making this criticism he forgets that he defined mutation in yeasts as " sudden genetic changes due to alteration in the molecular structure of a single gene or *alteration in the structure of an entire chromosome* " (*C. R. Lab. Carlsberg*, **24**, 1944, 79). How legitimate this criticism is could be seen from the proof offered by Roman, Hawthorne and Douglas in a recent paper (*Proc. Nat. Acad Sci., U.S.*, **37**, Feb. 1951, 79) that some of the spores investigated by them were actually diploid. " Meiotic aberrations " though they may not appeal to Winge may in fact be more common than imagined.

Ditlevsen (*C. R. Lab. Carlsberg*, **24**, 1944, 31) describes a gene governing cell form in *Saccharomyces italicus*. He states: " Of the four spores in each ascus, 2 formed short cell colonies, 2 long-celled " (p. 37). We have to remember that the important criterion on which Winge and collaborators distinguish " haploids " from " diploids " is cell size. In *Saccharomyces italicus* the cell size is governed by a gene. The short cell type is said to diploidize after a limited " haplophase ". When the diploid cell form itself is gene determined the only character on which a haploid could be differentiated from a diploid is the ability of the latter to form spores. But then, some of the diploids of Winge are asporogenous (Winge and Laustsen, *C. R. Lab. Carlsberg*, **22**, 1937, 114). Skovsted (*C. R. Lab. Carlsberg*, **24**, 1948, 250) admits that they had no morphological criteria to distinguish a diploid from a tetraploid.

When that is the situation one is surprised at Winge's criticism (1951) of our suggestion that in *Zygosaccharomyces* meiosis may occur in the vegetative phase. Considering the fact that what was described as endomixis in *Paramœcium* turned out to be a reorganization after a reduction division (Sonneborn, *Adv. Gen.*, **1**, 1947, 290) the suggestion is not so far fetched as Winge would have us believe. Winge states: " It is quite another matter, naturally, that *Zygosaccharomyces* can occasionally propagate itself vegetatively in the diplophase ". Winge and Laustsen (*C. R. Lab. Carlsberg*, **22**, 1939, 340) make the following statement regarding *Zygosaccharomyces priorianus*. " Thus we have to do with a type which is chiefly diploid, though partially haploid, and the latter fact of course involves its being referred to the genus *Zygosaccharomyces* ". Can we apply the same criterion to identify the progeny of the Spore IV described by Winge and Laustsen (1937, 111), which " resisted " all attempts to convert them into diploids, but which really became diploid six months later, as belonging to *Zygosaccharomyces* ?

The so-called species and genera in yeasts are identified on very nebulous criteria. Winge himself gives expression to this in the same paper where hybridization between the so-called genera is claimed. " An absolute identification of a yeast type under consideration, *e.g.*, Saccharomyces species, is generally impossible, even if it is often possible to refer it to some species already described " (*C. R. Lab. Carlsberg*, **22**, 1939, 344).

In view of the above statement one is surprised at his claim that because *Zygosaccharomyces* generally " form zygotes (asci) within the haploid clone ", they are " homozygous and immune against inbreeding " (Winge and Laustsen, **23**, 1940, 31). The *Zygosaccharomyces* species investigated by him was indistinguishable in its behaviour from that of *Saccharomyces*. But when it does not show any inbreeding degeneration the convenient assumption is of course brought out that *Zygosaccharomyces* are generally haploid !

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