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A CRITICAL EVALUATION OF THE QUESTION OF CYTOPLASMIC INHERITANCE IN YEASTS

BY

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1950

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INTRODUCTION

In two previous contributions (Subramaniam, 1950 *a*, *b*) it was indicated that a clarification of the contradictions that one comes across in the published literature on yeasts is imperative for any ordered advance in our knowledge. The earlier investigators (Winge, 1944; Winge and Roberts, 1948; Lindegren, 1945 *a*. *b*; Lindegren and Lindegren, 1946) have proceeded on the unsubstantiated assumption that polyploidy does not occur in yeasts and have tried to differentiate the so-called "haploid" from "diploid" yeasts purely on morphological criteria. Their investigations were on strains whose chromosome constitutions were unknown and naturally they had to depend on morphology.

A planned series of investigations on orthodox lines carried out in this laboratory indicated that induction of tetraploidy is easy (Subramaniam, 1945, 1947; Subramaniam and Ranganathan, 1948; Subramaniam and Krishna Murthy, 1949: Mitra and Subramaniam, 1949) and that it is possible to recover the diploid from the autotetraploid (Duraiswami and Subramaniam, 1950). In the light of the cytological evidence for the existence of polyploidy (Ranganathan and Subramaniam, 1948) it became apparent that in the absence of any criteria to distinguish "diploids" from "polyploids", Winge and Lindegren should have included both the above categories under their so-called "diploids". It follows as a corollary that their so-called

" haploids " should include " real haploids " as well as " polyhaploids ". Under the above circumstances, it was not at all surprising to find considerable disagreement between the workers regarding the criteria to be used for differentiating their so-called " haploids " from " diploids " (Subramaniam, 1950 *a*). When the fundamental assumptions are invalid that is what should be expected. The only apparently reliable character on which Winge identifies his diploids is the ability to form spores. But even Winge and Laustsen (1937, p. 114, Fig. 20, Pl. VI) record an asporogenous diploid. On the other hand, Lindegren and Lindegren (1946) remark: " One of our most interesting exceptions involved yeasts which were capable of producing ascospores directly in the round haploid cells without even passing through the stage of illegitimate copulation " (p. 128). Yeasts being polymorphic, the apparent reliability of using the only character, *viz.*, ability to form spores, to differentiate their so-called " haploids " from " diploids ", has in fact no validity in view of the above contradictory statements.

It is necessary to emphasize the above contradiction in order to show the unsubstantiated nature of another postulate. All investigators assume that a reduction division precedes spore formation. It should be obvious that when a so-called " haploid " sporulates, there cannot be any normal meiosis. Winge and Laustsen argue: " When 4 spores are present, it is rather improbable that some of them might in themselves be diploid, and indeed there is no evidence to suggest such a view " (1937, p. 104). On the other hand, Lindegren and Lindegren (1944) consider that homozygous diploids usually produce only unreduced ascospores which germinate and give rise to clones resembling the parent type (p. 209). But contrary to expectations, the spores of some of the homozygous diploids of Lindegern (1945 *b*, p. 120) gave a 2 : 2 gene segregation. It has to be emphasized that the postulate that meiosis precedes sporulation has no cytological confirmation and hence both Winge's claim that no unreduced spores can occur in a four spored ascus as well as Lindegren's claim that all viable spores in homozygous diploids are really diploids appear to be pure speculations.

When that was the case as regards the time of occurrence of meiosis in the life-cycle of yeast, it became apparent that the major classification of yeasts into " haplontic " and " diplontic " groups is arbitrary, artificial and unsubstantiated by valid evidence. A critical analysis of the criteria on which " haplontic " yeasts are separated from " diplontic " ones revealed that such a differentiation was based on another set of unsubstantiated assumptions. This unsatisfactory state of affairs necessitated the rejection of the invalid criteria and a re-evaluation on rational lines. It was shown

(Subramaniam, 1950 *b*) that 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 is the result of a reduction division in the vegetative condition, then, the shift from the *Zygosaccharomyces* to the *Saccharomyces* phase could be explained as the result of a simple gene mutation. Derivation of the life-cycles of *Debaryomyces* and *Nadsonia* is then an easy matter since such a reduction division may give rise to iso- or heterogametes. The above new interpretation (Subramaniam, 1950 *b*) was offered as an alternative and whether it is accepted or not, it demands serious consideration till such time as indisputable cytological data are available in regard to the real state of affairs. *Zygosaccharomyces* can show under particular conditions, the life-cycle said to be characteristic of the *Saccharomyces* species. Winge and Laustsen (1940) remark that the culture of *Zygosaccharomyces priorianus* derived even from a single spore "usually exhibits a mixture of haploid and diploid cells" (p. 30). They do not seem to have realized that a transformation from the *Zygosaccharomyces* to the *Saccharomyces* phase and *vice versa* demands a series of chromosomal and gene mutations occurring simultaneously (Subramaniam, 1950 *b*). In an earlier publication (1939) Winge and Laustsen state regarding *Z. priorianus*: "A certain amount of haploid growth, however, was observed both in the culture from Delft and in cultures produced from germinated single spores, and zygote formation was observed in the haploid colonies and subsequently spore formation when the yeast was transferred to blocks of plaster of Paris" (p. 340). If we consider that the "haplontic" yeasts are real haploids the fusion of vegetative cells should have produced a diploid. Usually in *Zygosaccharomyces* it is believed that the diploid phase is transitory and that it is immediately followed by meiosis and spore formation, the spores germinating directly. But Winge and Laustsen (1939) remark that the spores of the strain employed by them "germinated chiefly in the same manner as in *Saccharomyces* species, *i.e.*, now by spore copulation, now by haploid germination followed sooner or later by formation of twin zygotes or monozygotes" (p. 340). With the evidence at their disposal they conclude that the strain of *Z. priorianus* was chiefly diploid. But the above conclusion about its diploid nature did not deter them from offering the following explanation for the absence of any inbreeding degeneration. "*Zygosaccharomycetes* under normal conditions undoubtedly form zygotes (asci) within the haploid clone, which originates from the germination of a spore. Consequently they are homozygous and immune against inbreeding. *Zygosaccharomycetes* might

scarcely exist without possessing immunity from inbreeding" (Winge and Laustsen, 1940, p. 31). Thus, inbreeding degeneration is said to be absent in *Zygosaccharomyces* owing to its immunity and is equally lacking in *Saccharomyces validus* because "its chondriosomes divide slightly more simultaneously with the nuclear division than those of *S. cerevisiæ*" (p. 37).

Our results together with the theoretical difficulties in accepting the existence of an inbreeding degeneration (Duraiswami and Subramaniam, 1950) led to an analysis of the Cytogene and Plasmagene theories which appear really to be off-shoots of the claim of inbreeding degeneration in yeast by Winge and Laustsen (1940). *The primary question is whether there is any inbreeding degeneration at all*?

Direct Diploidization and Inbreeding Degeneration

According to Winge and Laustsen, the vegetative form could originate (1) by fusion of two spores, (2) fusion of a cell resulting from the germination of a spore with an ungerminated one, (3) fusion of "haploid" cells originating from different spores, (4) fusion of "haploid" cells from the same spore and (5) fusion of the two nuclei originating by the initial division of the spore nucleus. Genetically the last two classes should be indistinguishable. But they observed differences in the germinating power of the spores produced by vegetative cells originating by cell fusion and nuclear fusion. The spores produced by the form arising from cell fusion were viable while those in cultures said to originate by nuclear fusion were not. This difference in the viability of the spores in strains which are homozygous and genetically identical is suggested to be the result of an inbreeding degeneration and an explanation is offered that this may be due to a deficiency in the complement of the chondriome in the two strains. Thus another unknown quantity is brought into the field where confusion has been the order of the day. For about ten years, I had carried out a series of investigations on the Golgi Apparatus and Mitochondria in animals ranging from Protozoa to Vertebrates, with special reference to the probable role of these cytoplasmic inclusions in cell metabolism (Subramaniam, 1934, 1937, 1939). Viewed with that background it appeared that an explanation of inbreeding degeneration based on deficiency of mitochondria was, to say the least, untenable. Alternative interpretations were, therefore, sought for.

The whole question of inbreeding degeneration centres round the problem of direct diploidization. *Is there a nuclear fusion after all*? A critical analysis of their evidence is revealing. Demonstration of nuclear fusion succeeding spore fusion being itself difficult, it is admittedly much more arduous to present critical evidence for the fusion of the two nuclei formed

by division of the spore nucleus. Winge and Laustsen (1937) illustrate in their Fig. 5 four binucleate spores. They comment: "There can hardly be any doubt that such pictures show a stage of diploidization, which will be accomplished indeed, when the two nuclei fuse; but as a matter of course, it cannot be proved definitely that the two nuclei will fuse later on" (p. 105). Two of the spores in their Fig. 5 are budding and the bud in one is as big as the spore itself. The presence of buds makes one sceptical regarding the fusion of the nuclei. The binucleate stage may legitimately be interpreted as the division of the spore nucleus preceding bud formation. A similar stage is illustrated in the Pictographic Summary of the mitotic cycle published for the two-chromosome brewery yeast (Subramaniam, 1946).

Realizing the difficulty of offering critical proof for "direct diploidization" they remark: "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. This is quite evident from the fact that elongated cells form ascospores directly when they are placed on plaster block, whereas round cells cannot be transformed into asci without pairwise fusion of the cells—that is, as if the yeast belonged to the *Zygosaccharomycetes*" (p. 105). Thus we have an interesting situation. Whereas there has been disagreement among the investigators regarding the criteria on which "haploids" could be identified, the evidence adduced for "direct diploidization" has itself not been critically assessed.

In their paper on inbreeding degeneration (1940) they repeat with greater emphasis that "it is possible both (1) to distinguish haploid yeast types from diploid ones from the shape and size of the cells; (2) to demonstrate that haploid types are unable to form spores without diploidizing; and (3) to obtain cytological confirmation of this peculiar way of diploidization" (p. 22). From the quotations given from their earlier paper, the cytological confirmation is of questionable validity. In 1937 they obtained from the same Danish Baking Yeast a diploid (Winge and Laustsen, 1937, Pl. VI, Fig. 20) which was incapable of sporulation (p. 114). Thus even some diploids are incapable of sporulation and hence their identification of "diploids" is based purely on shape, size and mode of budding of the cells. Guilliermond (1920) emphasizes that yeasts are polymorphic and may "depending upon circumstances, take on variable forms temporary or permanent" (p. 179). *A vital factor not taken into account by them is polyploidy.*

The moment we consider their starting strain to be a tetraploid, a very rational explanation is possible of the so-called "inbreeding degeneration" on the basis of tetraploidy and diploidy. It must be mentioned here that

diploid spores of tetraploid strains can have balanced chromosome constitutions, and these germinating directly can in turn produce spores, which would have only a haploid chromosome complement. Thus, while the spores produced by the tetraploid are capable of unlimited proliferation, the unbalanced haploid spores can at best produce only a few cells by direct germination. Winge and Laustsen (1940) base their speculations on inbreeding degeneration *only on the power of germination of the spores.* We have to remember that even spores which were incapable of direct germination were capable of fusing with other spores and giving rise to a vegetative generation. A rational explanation is presented below for their so-called "inbreeding degeneration". (Text Fig. 1).

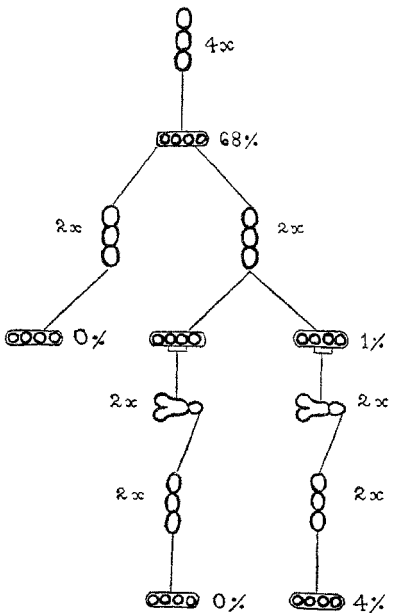

FIG. 1

TEXT-FIG. 1. Fig. 9 of Winge and Laustsen (1940, p. 26) modified to offer an interpretation for the so-called "Inbreeding Degeneration". Diploid spores alone show a high percentage of germination,

Based on the above, it is not at all surprising that regeneration of the germinating power of spores was not possible in those strains originating from fusion of spores which themselves lacked the power of germination. The confusion is entirely due to the following two unsubstantiated assumptions, *viz.*: (1) the original strain is a diploid and (2) that parthenogenesis does not occur. It has been demonstrated in this laboratory that polyploidy is more common in yeasts than imagined by many (Subramaniam, 1945, 1947; Subramaniam and Ranganathan, 1948; Mitra and Subramaniam, 1949). Diploid spores of autotetraploids should be capable of parthenogenetic development. A simple straightforward cytogenetic explanation is thus possible of the so-called "inbreeding degeneration" without bringing in such unknown quantities as the chondriome or the cytoplasm.

The Cytogene Hypothesis

It is therefore apparent that not only the possibility of polyploidy in yeasts has been ignored, but that the "haploids" and "diploids" have been identified on questionable criteria. One can hence appreciate the soundness of the foundation for radical theories on heredity. The Cytogene Hypothesis of Lindegren does not, therefore, stand even the first touch of an objective analysis.

In "Mendelian and Cytoplasmic Inheritance in Yeasts" (Lindegren, 1945 *b*) he suggests: "Since *S. carlsbergensis* is homozygous for two pairs of genes which produce melibiozymase, there are four loci in the diplophase of this organism capable of producing this enzyme. There are four corresponding recessive alleles in *S. cerevisiæ*, which is probably the most cosmopolitan and best established yeast species" (p. 119). *The four loci may be in a tetraploid and not in a diploid.* It is such a strain of *S. carlsbergensis* which is hybridized with commercial strains of *S. cerevisiæ* which are also in all probability polyploids. The resulting hybrids appear, therefore, to be really polyploid hybrids. If this suggestion is considered probable, then Lindegren's analysis based on the supposition that the hybrid is a diploid is necessarily invalid. Lindegren and Lindegren (1946) first hybridized (*a*) supposed diploid strains of *S. carlsbergensis* and *S. cerevisiæ*; (*b*) the spores of such a hybrid were assumed to be "haploid" and were used for back crossing. Two series of observations gave entirely different results. In the first experiment, "one ascus in ten produced a 1 : 1 segregation", while in the second, "sixteen of eighteen asci gave 1 : 1 ratios" (p. 117). On the basis of the first experiment they concluded that there are "two nonallelic genes capable of controlling the fermentation of melibiose", but considered in the light of their second experiment that the same *S. carls-*

bergensis " carries only a single M gene ". The unusually large number of fermenters in the earlier experiment was taken to indicate " that many of the recessive alleles were ' masked ' by the acquisition of the dominant phenotype " (p. 117). The initial questionable assumption that the strains are diploid necessitates a further series of postulates to explain why recessives appear in an intra-ascus cross of spores showing the dominant phenotype. It is on such a series of unsubstantiated criteria that radical views are offered. " In the occasional exceptions, three or four of the spores in an ascus were fermenters, indicating that some of the expected recessive (non-fermenting) progeny had acquired the ability to ferment. These " masked " recessives acquired the factor controlling fermentative ability (apparently at meiosis) and retained it in the absence of the respective substrates, galactose and melibiose ". They conclude: " No simple scheme involving a cytoplasmic or a genetical mechanism could be invoked to explain this phenomenon " (p. 123). Lindegren and Lindegren considered only probabilities based on inhibitory genes and incompatible cytoplasm, *but never took into consideration the probability that they may be dealing with a polyploid hybrid and not a diploid one.*

We have to remember Little's (1945) comment that one of the " fundamental concepts underlying diploid genetics, that of " purity of gametes " is meaningless in the discussion of tetraploid genetics " (p. 60). There are five genotypes possible. " Often when two alleles are present in equal proportions, one is completely dominant over the other, but it may not be capable of completely masking the effect of three successive genes in a simplex hybrid. For this reason incomplete dominance is more common in tetraploids than in diploids " (p. 78). If one proceeds to analyse segregation in polyploids assuming them to be diploids, results capable of a rational interpretation would appear unique. Some observations on *Primula* are reminiscent of Lindegren's " masked " recessives. In diploids, green stigma (G) is dominant over red (*g*). " In tetraploids, on the other hand, plants with the constitution *Gggg* have green stigmas, but the " flowers have a darker shade, in some cases nearly as dark as that of the pure recessive form ". In addition, the factor B for magenta flower colour is completely dominant over red in diploid but in the tetraploid class *Bbbb* " exhibits colours varying from magenta to almost pure red " (Little, 1945, p. 78).

If we consider the strains employed by Lindegren and Lindegren (1946) to be tetraploids, the genotype of the hybrid may be duplex. When selfed it may produce a 15 : 1 (Gregory), or 35 : 1 (Muller) or 21 : 1 (Haldane), or 77 : 4 (Mather) ratio (Little, 1945, p. 63). Lindegren and Lindegren carried

out such a selfing. In their Table 3. Hybrid I was obtained by mating *S. cerevisiæ* (*m*) with *S. carlsbergensis* (M). The spores A and D in the first ascus of the hybrid said to belong to the dominant phenotype were crossed to obtain Hybrid III. Analysis of sixasci of Hybrid III gave an 18 : 2 ratio. "The unfixed nature of tetraploid ratios introduces problems in statistical analysis not encountered in dealing with diploid ratios". "The important statistical problem is, therefore, not to determine which ratio the data fit more closely, but to find out to what extent the two opposing forces of reductional and equational separation have affected the data" (Little, 1945, pp. 79–80). Any clear analysis of tetraploid segregation necessitates examination of large populations. "For example, it is necessary to have a population of at least 1,700 plants in order to be certain that any ratio obtained can be proven to deviate significantly from either a 35 : 1 or a 21 : 1 ratio" (p. 80).

Judged on this basis the data presented by Lindegren and Lindegren (1946) as well as Winge and Roberts (1948) are too scanty for drawing any valid conclusions. It is curious to find Cytogenes being defined differently in two publications in the same year. In the earlier one (1945 *b*), adaptive enzymes are called "Cytogenes" (p. 121), while in the later contribution (1946) it is stated: "The ribose nucleoprotein carrying the Cytogene in the cytoplasm is the *precursor* of the enzyme, which gives the enzyme its specificity in the presence of the specific substrate" (Lindegren and Lindegren, 1946, p. 126).

The speculations of Lindegren on the "Cytogene" while highly interesting to read, have little experimental evidence to justify any serious consideration.

The Plasmagene Hypothesis

Spiegelman (1946) identifies the nucleoprotein in the cytoplasm, instead of the enzyme, as the Plasmagene owing to its similarity to the gene and since it is said to be derived from the gene. The Plasmagene according to him "is a more or less complete gene replica, which possesses to a varying extent the capacity to self-duplicate. It is not a special or unique cytoplasmic component in the sense that it is outside normal physiological processes. It is an integral part of the enzyme-synthesizing system and is the normal link by means of which genes can effect control over protein formation in the cytoplasm" (p. 273).

These conclusions are arrived at by an elegant series of experiments which unfortunately are based on inaccurate assumptions. The fatal objections presented against Lindegren's Cytogene hypothesis apply in their

entirety to Spiegelman's (1946) conclusions also since his experiments are on the identical hybrids discussed by Lindegren and Lindegren (1946), but in a different direction. He assumes (1) that *S. carlsbergensis* and *S. cerevisiæ* are " diploids ", (2) that their spores are " haploid " and (3) that the hybrids obtained are " diploids ". The hybrid obtained by mating the spores of *S. cerevisiæ* with that of *S. carlsbergensis* was phenotypically positive. " Out of 6 asci (of the above hybrid), from each of which all four spores were recovered and tested. 3 behaved like the original *S. carlsbergensis* yielding all 4 segregants as positive; 2 asci yielded 3 positives to 1 negative; and 1 ascus produced the 1 : 1 ratio of positives to negatives expected of a heterozygote segregating a single dominant gene " (p. 257). Spiegelman considers two plausible alternative interpretations. " Only one gene actually segregates, but the expected 1 : 1 Mendelian ratio is obscured by cytoplasmic components originating from the positive spores, which can form the enzyme in the absence of the gene ". " Two or more genes either one of which can mediate the formation of melibiase, are segregated " (pp. 257–58). The acceptance as an undisputed fact that the cultures used in hybridization are " diploids " makes Spiegelman veer to the cytoplasmic self-duplication hypothesis. It is problematical whether Spiegelman or Lindegren would have ventured on an elaboration of the cytoplasmic self-duplication hypothesis, if existence of polyploidy was considered probable. Under the circumstances the hypothesis that segregation of two or more genes capable of producing melibiase requires serious consideration.

The adaptation experiments are carried out with spores from a back cross hybrid (Hybrid IV) produced by mating a positive spore of Hybrid I with the negative one of *S. cerevisiæ*. It was indicated before that in tetraploids one cannot be sure of the " purity of the gametes ". If enzyme synthesis can proceed in the absence of the gene which initiated it, Spiegelman (1946) believes that elimination of the gene initiating production would be critical proof for the existence of cytoplasmic factors. He considers that the back-cross Hybrid IV is suitable for the above purpose since a regular segregation has been observed. " Under such conditions one could be relatively certain that only two out of every four spores carried the gene responsible for the fermentative capacity " (p. 259). This assumption becomes invalid the moment we consider that the parents as well as the first hybrid may be polyploids.

If the parents are tetraploid, the spores of the hybrid may be of three types: MM, M*m* or *mm*. The observations of Spiegelman (1946) and Lindegren and Lindegren (1946) indicate such a possibility. In his experi-

ments on segregation in the presence of melibiose he found (Spiegelman, Table 4, p. 259) that while all the four spores in the first 6 asci were positive, 2 of the spores in the 7th ascus were negative even in the presence of melibiose. Thus even among the so-called recessives, there are two types: (*a*) those which adapt and (*b*) those which do not. Is the latter the real recessive ?

Lindegren and Lindegren (1946) consider the first type as the " masked " recessive. They state: " Recessives that are masked by continued exposure to substrate during meiosis and subsequently unmasked by dissimilation may carry a factor controlling adaptation in the cytoplasm; while recessives that acquire the dominant character at meiosis may perpetuate the ability by some chromosomal mechanism (p. 123). But that is not all. They found a " relatively high frequency of 3 : 1 ratios resulting from back-crossing a ' masked ' recessive to a true recessive " (p. 124). All these results would be comprehensible when it is remembered that five genotypes are possible in tetraploids.

The argument that while the back-cross (Hybrid IV) gives regular Mendelian segregation, the first hybrid does not is explained by Spiegelman as the result of two dilutions of the *S. carlsbergensis* cytoplasm by that of *S. cerevisiæ*.- " The cytoplasmic factors that obscure the Mendelian picture of Hybrid I have thus apparently been diluted in Hybrid IV to the point where they can no longer play critical roles in determining potentiality for melibiase formation " (p. 261). Such a back-cross may as well make a " duplex " into a " simplex " hybrid.

A New Mode of Gene Action

The confusion regarding the criteria for distinguishing the " real " recessives from " masked " recessives indicate that only general alternative explanations could be offered for adaptation. The ability to ferment a particular sugar depends on the possession of the specific gene and when a strain possesses genes capable of fermenting different sugars, the substrate determines as to which gene should function. The rate of fermentation appears to depend on the gene dosage in a peculiar way (Mitra and Subramaniam, 1949). Wager and Peniston (1910) and Guilliermond (1920) considered that fermenting cells are comparable to secretory cells of higher organisms. The vegetatively dividing cells of *Drosophila* have the diploid chromosome constitution. In the salivary gland, on the other hand, the cells have endopolyploid nuclei. Similarly, yeasts become endopolyploid as a prelude to fermentation. Though endopolyploid cells may divide occasionally or regularly, the change to endopolyploidy is an irreversible

one and their eventual fate is death and disintegration. Taking the salivary gland as an example, the cells in such a gland are capable of producing only the particular secretion and after one or more secretory cycles disintegrate and are replaced. The genes for the different functions which are present in the zygote come into action only in specific tissues. Once differentiation is completed the particular genes alone are active, the rest being inactive.

Logically one has to conclude that the basic steps in fermentation are carried out by gene complexes and that the individuals of the complex have very low mutation frequencies since inactivation of one member of the complex would throw the mechanism out of gear. It would not be surprising if they are " heterochromatic " and occur in repeats. The genes said to initiate the production of melibiozymase and galactozymase really govern only master reactions. When we remember that in *Bonellia viridis* the sex could be determined by the pH of the medium, the effect of the substrate in adaptation in yeasts should merely affect the master reaction.

There is a much more vital consideration to be kept in view. In particular tissues, most of the genes unconnected with the function of that tissue are inactive. The salivary gland cells of *Drosophila* are endopolyploid. When the chromosomes are duplicated, it follows that the gene number is also automatically duplicated. Can we not legitimately conclude that the gene complexes governing secretion of saliva function probably only when duplicated above a basic number ? Since most of the tissues are endopolyploid to varying degrees, this mode of gene action may be more common than imagined. This would obviate the difficulty of geneticists in finding a rational explanation of growth and differentiation in terms of the orthodox concept of the gene. The enormous amount of work on morphogenesis has produced acceptable interpretations of the ordered process of tissue and organ differentiation based on organizers and evocators (Needham, 1942). The difficulties in interpretation arose only when the nuclei of tissues were assumed to be diploid. " Thus, the assumption that every time a new protein molecule is formed during growth the gene on the chromosome must intervene as a kind of model implies that growth must proceed linearly from a relatively minute portion of the cell. The kinetics of cell growth follow an autocatalytic law and so are not consistent with this " (Spiegelman, 1946, p. 272). In the above discussion by " growth " is meant " the increase in the amount of active protoplasm " said to be characterised " especially by increase in protein " (Wright, 1941, p. 500). When we consider that secretory cells are highly endopolyploid, the above objections automatically disappear. The production of protein molecules is not by a very small

number of genes but by a very large number of identical genes in the endopolyploid nucleus. This would remove the necessity for postulating the existence of gene replicas in the cytoplasm to explain the exponential increase. When genes have been duplicated during endopolyploidy, a self-duplicating mechanism in the cytoplasm becomes superfluous.

It has to be understood very clearly that polyploidy and endopolyploidy are not synonymous. Polyploids like diploids can become endopolyploid. For example, our diploid and tetraploid yeast strains become endopolyploid during fermentation. The duplication of the chromosomes does not result in a doubling of the alcohol output but only affects the rate of production. The acceleration of the rate of production in the case of the tetraploid could be explained on the basis of endopolyploidy. If a cell becomes fermentative only when it is 16-ploid, whereas the diploid would reach that stage after three duplications, the tetraploid would require only two. The rate of fermentation by the tetraploid cells is naturally quicker because they take less time to reach that minimum stage of endopolyploidy.

Ciliates and Cytoplasmic Inheritance

On the basis of the above considerations, are we entitled to interpret Sonneborn's (1947) remarkable observations on *Paramecium aurelia* in a similar manner? Are some of his varieties polyploid? The cytoplasmic factors occur only in varieties of Group B. In his Group A "cytoplasmic factors have not been found at all; the characters seem to be directly controlled by the genes without detectable intermediacy of cytoplasmic factors" (p. 327). Sonneborn himself remarks: "Chen believes his stocks constitute a polyploid series which has arisen as a result of rare abnormalities in the process of conjugation. Diverse chromosome numbers are even found in different stocks of the same mating type" (p. 333–34). That is in *Paramecium bursaria*. The evidence for the presence of cytoplasmic factors in *P. aurelia* is presented on the belief that it is a diploid. Hertwig believed that the diploid chromosome number varies between 8 and 10. Diller and Sonneborn consider that it is between 30 and 40 (Sonneborn, 1947, p. 276). If Hertwig's observations are correct then, Diller and Sonneborn should have been investigating polyploids. When polyploidy is considered possible in *P. bursaria* there is no reason why it cannot occur in *P. aurelia*.

Is the difference in the behaviour of his Groups A and B merely one of diploidy and polyploidy? If that is so, a very simple alternative interpretation is possible. We have to remember that the genes in Ciliates become functional only when duplicated above a basic number (Mitra and Subramaniam, 1949). When the number of "killer" genes get reduced as a

result of a mating with a pure " sensitive ", their dosage in the endopolyploid macronucleus may not be sufficient for the initiation of activity. Transfer of " killer " cytoplasm merely stimulates these genes to activity. This would be in conformity with adaptation to galactose fermentation in yeasts and determination of sex in *Bonellia viridis*. Acceptance of the above explanation would render unnecessary the belief " that the gene in Group A is composed of two parts, one comparable to the gene of Group B and the other comparable to the cytoplasmic factor of Group B " (Sonneborn, 1947, pp. 327–28).

Conclusion

Cytoplasm may play as yet an unknown role in heredity, but the availability of alternative cytogenetic interpretations render superfluous and unnecessary any necessity for postulating the existence of self-duplicating organelles in the cytoplasm. Our knowledge of the cytology and genetics of yeasts is still in a confused state. Investigations on yeasts, therefore, cannot be a basis for unorthodox theories or sweeping generalizations. Nor is blind acceptance of such concepts desirable or necessary. When artistic superstructures collapse for want of strong foundations, it would be the better part of wisdom to build up strong foundations before planning ambitious superstructures.

Summary

1. In the light of the cytological evidence for the existence of polyploidy in yeasts, it became evident that in the absence of any criteria to distinguish diploids from polyploids, investigators on the genetics of yeasts should have included both the above categories under their so-called diploids. It follows as a corollary that their so-called haploids should include real haploids as well as polyhaploids.

2. A simple cytogenetic explanation is possible for the so-called in-breeding degeneration observed by Winge and Laustsen without bringing in such unknown quantities as the chondriome or the cytoplasm. If we consider their starting strain to be a tetraploid, the spores can have balanced diploid chromosome constitutions. These can germinate directly and produce in turn spores which would have only a haploid chromosome complement. While the spores produced by the tetraploid would be capable of unlimited proliferation, the unbalanced haploid spores can at best produce only a few cells by direct germination.

3. The curious genetic segregations observed by Lindegren and Spiegelman, which formed the basis for the "Cytogene" and " Plasmagene " theories, find a rational explanation on the basis of polyploid segregation.

The possibility of polyploidy in yeasts has been ignored and the so-called haploids and diploids have been identified on questionable criteria. Radical theories on heredity should be based on accurate facts to warrant any consideration.

4. A new mode of gene action is elaborated. The genes for the different functions which are present in the zygote come into action only in the specific tissues. During differentiation, different tissues become endopolyploid to varying degrees. Specific genes come into action only when duplicated above a basic number. Once differentiation is completed only the particular genes are active, the rest being inactive. Such an explanation would obviate the necessity for postulating the existence of gene replicas in the cytoplasm to explain the exponential increase in protein during growth and secretion. This interpretation would offer a new approach to the problem as to how during ontogeny specific genes become functional in the respective tissues.

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Note added in proof.—Experimental evidence justifying the validity of the alternative interpretation offered in this paper for the so-called inbreeding degeneration claimed in yeasts by Winge and Laustsen (1940) is afforded by the observations of Roman, Hawthorne and Douglas (*Proc. Nat. Acad. Sci.*, U.S., **37**, 1951, 79). One of the asci obtained from a cross between two clones of *Saccharomyces* exhibited an irregular ratio. This has led them to consider that the original clone from which the ascus arose should have

been a tetraploid. The diploid spores from the above asci germinated directly and produced in turn spores which gave a 2 : 2 segregation. Winge (*C. R. Lab.*, Carlsberg, **25,** 1951, 85) belittles such a suggestion by Duraiswami and Subramaniam (1950) and comments that the classification of the haploids into "real haploids "and " polyhaploids " has *actually no significance* and is of only terminological interest. We are rather surprised at this statement. The concept of the " purity of gametes " is inapplicable to polyploids and the above classification should have a much wider significance than imagined by Winge.

The same phenomenon is interpreted by Fowell (*J. Inst. Brew.*, **48,** 1951, 180) in a different manner. Out of the 18 " haploid " strains of the D. C. L. Baker's yeast, three gave 20 to 30% sporulation and in one, 60% of the asci were 4-spored. Fowell, following the convention set up by Winge, tries to argue that the sporulation is really by haploids. As shown in the review on the problem of haploidy in yeasts (Subramaniam, 1950), there is no consistency in the criteria for the identification of the " haploids ". When haploid cultures show *unusually large cells* these are still considered by Fowell to be haploid because they *do not sporulate*. On the other hand, when haploid cultures *do sporulate*, it is argued that they have to be identified as haploids because their cells are *smaller in size*. From the foregoing examples it is patent that the primary classification into " haploids " and " diploids " is based on morphological criteria of questionable validity. Fowell's record of the occurrence of " unusually large vacuolated cells " in haploid cultures should be a sufficient answer to Winge's (1951) claim that haploids, diploids and tetraploids should show progressive increase in volume. His criticism of our work based on such theoretical possibilities is not only unjustified but unwarranted in view of his own admission (Winge and Laustsen, 1937, p. 113) that cell size in yeasts cannot be considered a suitable criterion for genetic analysis.

Acceptance of Fowell's observation that some haploids can sporulate and that many of the asci are 4-spored, undermines Lindegren's claim that only legitimate diploids do produce a high proportion of 4-spored asci and Winge's claim for direct diploidization and the resulting inbreeding degeneration. As shown in this paper, the evidence presented by Winge does not justify the claim of a direct diploidization.

Unfortunately Fowell's results do not entitle one to believe that it is the " real haploids " that have sporulated since cytological evidence is lacking. Meiosis is considered to be irregular in haploids and when they do produce viable germ cells, the resulting progeny from selfing are all either diploids

or polysomics (Darlington, 1937). How unreliable is the assumption that normal *meiosis* precedes sporulation could be made out from the claim that "haploid" cultures do produce 4-spored asci!

The observations of Fowell that the formation of a zygote need not necessarily be followed by the fusion of nuclei and that these dicaryotic zygotes bud off haploid cells from any part of the zygote, "even from the bridge connecting the component cells", emphasizes the urgent need for confirmatory cytological evidence to justify their acceptance. It would be well to remember that Fowell's claim that some of the zygotes are dicaryotic is as unsubstantiated by critical cytological evidence as Winge and Roberts' (*Nature*, **165**, 1950, 157) claim of an extra mitosis to fit their theoretical assumptions to observed irregular ratios.

It is rather surprising that while the criteria for the differentiation of haploids from diploids are full of contradictions, there is considerable agreement among the students of yeast genetics as to their validity! Whereas all of them speculate on cytological probabilities which seem to have little justification except that they conveniently explain otherwise inconvenient irregular genetical behaviour, they repeatedly emphasize "the deplorable lack of agreement about the identity of chromosomes in yeasts" (Fowell, 1951, p. 195). While explanations based on cytological observations are dismissed as "fantastic", Winge (1951) does not seem to realize that such criticisms are more applicable to the speculations in the special field in which he has taken a leading part. The modern work on yeast genetics appears to be characterised by the fact that while theories are put forward to explain certain apparently peculiar results, these same experimental results are presented afresh as support for the theories propounded. When inconvenient but legitimate criticisms are offered, they are distorted to produce the question: "Is this a justifiable form of Science!"

In all this welter of confusion, it is rather refreshing to read Fowell's conclusion (p. 195) that in the absence of *vital information* on the cytology of yeasts, "it must be premature to dismiss all the conventional explanations for the irregular segregation ratios, and even more premature to elaborate unorthodox theories about gene structure and behaviour". It is this identical point of view which we have been urging on the workers in the field. A knowledge of the cytology of yeasts should precede and not follow investigations on the genetical side.

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