STUDIES ON THE CYTOLOGY OF YEASTS

VI. Lethal Chromosomal Mutations in Yeasts

BY B. RANGANATHAN, M.Sc., PH.D.
AND
M. K. SUBRAMANIAM, M.A., D.Sc., F.A.Sc.
(Cytogenetics Laboratory, Department of Biochemistry, Indian Institute of Science, Bangalore-3)

CONTENTS

SUMMARY

1. The fate of the cells originating as a result of mitotic aberrations in the distillery yeast has been investigated. Yeasts appear to be more favourable for a study of the chromosome lethals, since changes in a growing population could be observed at very short intervals.

2. Some recent publications are critically reviewed and it is shown that the cause for confusion is the result of a lack of appreciation of the important differences in the cytological behaviour of aerobically growing cultures from those in active fermentation.

3. Abnormal segregations of chromosomes in the hexaploid, pentaploid and triploid cells are described. Amitosis, lagging of chromosomes, micronuclei formation and pycnosis appear to be more frequent in the hexa- and pentaploids than in triploids. A segregation of chromosomes without splitting occurs in all the three forms.

4. Regular mitoses occur in the diploid immediately after its origin by mitotic irregularities in the various stable and unstable polyploid cells. Since stable diploids could not be isolated by serial dilution plates it appears that they transform themselves into tetraploids by "somatic doubling".

5. Possession of two or four chromosomes does not mean that these should be viable combinations. When cells apparently having chromosome numbers suggestive of viability show micronuclei formation,

it appears that different pairs of chromosomes in the original tetraploid may not have identical gene sequences.

6. A pictographic summary of the mitotic irregularities and the fate of the various types originating as a result of such aberrations is given.

7. Mitotic aberrations appear to be under genetic control. Cells with unbalanced chromosomal complements attempt to produce the original four chromosome condition by irregular segregation of the unsplit chromosomes. This may in all probability be a regulating and stabilising mechanism to compensate the lethal effects of mitotic abnormalities. A similar mechanism probably operates in endopolyploid cells also.

8. If tetraploidy itself is the result of a gene mutation, the same mutant allele in the diploid originating from a tetraploid should produce a somatic doubling.

9. When purity of gametes is meaningless in polyploids, the peculiar cytological behaviour of the auto-tetraploid distillery yeast emphasizes the need for caution in offering radical theories to explain some curious segregation observed in hybridization experiments.

INTRODUCTION

The occurrence of mitotic aberrations in the distillery yeast (Ranganathan and Subramaniam, 1948) naturally gave an impetus to a study of the fate of the various cells having different chromosome numbers. The irregular distribution of the daughter chromosomes during division resulted in the formation of cells having two, three, five and six chromosomes in addition to those with the normal number, four. It was suggested in that paper (Ranganathan and Subramaniam, 1948) that the distillery yeast may be an auto-tetraploid and that the presence of mutant genes may be responsible for the chromosomal aberrations.

If, as indicated, the distillery yeast is a naturally occurring auto-tetraploid, it followed logically that the diploid should have a balanced chromosome complement and hence may be capable of isolation. With this end in view the yeast strain was repeatedly plated out at periodic intervals for over a year. Cytological investigations of the cultures isolated from agar plates showed identical pictures. This suggested the possibility that cells having two chromosomes may either have died out or reconstituted the original number by a somatic doubling. Cell types having three, five and six chromosomes were unbalanced and hence lethal. But even such lethal combinations gave indications of possible mechanisms as to how the original tetraploid condition could be reconstituted. This discovery was so surprising that the inter-

pretation of the results had to be held up for three years (Subramaniam and Ranganathan, 1946 *a*, *b*) in order to clearly differentiate such phenomena in aerobic cultures from those occurring in fermenting cells. Investigations on a two chromosome brewery strain (Subramaniam, 1946, 1948 *b*, 1951 *a*, 1951 *b*, 1952; Duraiswami and Subramaniam, 1951) indicated that during active proliferation under aerobic conditions, the cells divide mitotically and even show centrioles with centrospheres (Ranganathan and Subramaniam, 1947); but the same strain during fermentation presented an entirely different picture. When cells in a five-day old fermenting culture were stimulated to divide by addition of fresh wort, the cells showed a variety of stages (Subramaniam, 1948 *b*) suggesting that they had become endopolyploid during fermentation. This necessitated a standardisation of the conditions for a study of the nuclear behaviour in yeasts (Subramaniam, 1948 *a*; Duraiswami and Subramaniam, 1951). A loop of yeast inoculated into a fresh tube of wort would multiply mitotically for some time, but with the progressive decrease in the amount of oxygen, there would be a gradual increase in the number of fermenting, endopolyploid cells. The role of endopolyploidy in cell differentiation is itself little understood in the case of higher organisms. The cytological changes that accompany a shift from the aerobic to the anaerobic type of metabolism in yeast is bristling with various unsolved problems (Prahlada Rao and Subramaniam, 1952 *a*, *b*). It became necessary, therefore, to distinguish clearly the fate of the chromosome lethals originating as a result of mitotic aberrations from those arising by the division of endopolyploid cells. When repeated examinations of aerobic cultures showed identical cytological pictures and when cells showing mitotic irregularities appeared side by side with chromosome lethals attempting to divide, it became obvious that the behaviour of such lethals is absolutely unrelated to that of the products of division of endopolyploid cells. This was confirmed from an entirely different angle. If the distillery yeast is an auto-tetraploid, and if the mitotic aberrations are the result of auto-tetraploidy, then similar phenomena should be observed in artificially induced auto-tetraploids. In fact, the same mitotic irregularities occur in an auto-tetraploid brewery yeast (unpublished observations) obtained by treating the two chromosome brewery control strain with acenaphthene (Subramaniam, 1945, 1946, 1947, 1951 *b*; Subramaniam and Ranganathan, 1948).

The above confirmation together with the numerous records of comparable chromosome aberrations in higher plants (Vaarama, 1949) suggested a presentation of the following interesting observations. These have another unusual interest. In higher plants it is difficult to follow the fate of the

products of mitotic abnormalities. In yeast, on the other hand, it is relatively easy to observe the changes in a growing culture at very short intervals. Therefore, if preparations made at a specific period show mitotic abnormalities, examination of the culture again after a short lapse of time should give one an idea of the fate of these chromosome lethals. Yeasts, thus appear to be much more favourable for an investigation of the behaviour of the chromosome lethals than higher plants. Apart from these considerations, mitotic aberrations are interesting by themselves, because of the suggestion of Skovsted (1948), *unsupported by any cytological evidence*, that the various types observed by him after camphor treatment may be the result of cytological irregularities !

A CRITICAL EVALUATION OF SOME RECENT PUBLICATIONS

An elaborate review of our knowledge of the cytology of yeasts has already been published (Ranganathan and Subramaniam, 1948). It was stated there that much of the confusion in this field could be traced to the selection of fermenting cells for cytological investigations. In this connection, it would be desirable to remember the following statement of Guilliermond (1920): "The structure of yeasts is easy to interpret at the beginning of development. During the active period of fermentation they become more complex. What complicates the subject at this moment is a very active secretive action. Like all secreting cells they present a series of cytological phenomena in connection with secretion" (p. 46). At the time Guilliermond made the above observations, the phenomenon of endopolyploidy was still unknown. Darlington (1937) while referring to the behaviour of secretory glandular tissues of animals remarks that mitosis need not generally occur in them. When mitosis is excluded, "the nucleus loses its ordinary property of perpetuating a characteristic complement of chromosomes and may become more or less highly specialised for its immediate physiological functions" (p. 175). The chromosomes in the salivary glands of Diptera are enormous in size. "They are about a hundred times as long as metaphase chromosomes." From the above observations, it is apparent that in higher organisms it is only on the basis of a critical study of normal mitotically dividing cells that a rational explanation could be offered for the changes accompanying endopolyploidy. Similarly in yeasts it is possible to interpret the alterations occurring in fermenting cultures only after a thorough study of the cytological behaviour of actively proliferating cells.

The suggestion of Skovsted (1948) that the various mutations induced by camphor in yeast may be the result of cytological abnormalities is based

on the opinion of Levan (1947) that yeasts should have at least ten chromosomes. It becomes necessary, therefore, to examine how far Levan's conclusions could be accepted. A careful perusal of his paper revealed the fact that no attempt was made by him to investigate separately the cytological behaviour of his strain during aerobic growth and during fermentation. It is without any such basic investigations that he has tried to evaluate the effect of various chemicals on his strain of yeast. That the physiology of the yeast cells is highly important is not realised, for, it is only at the end of the paper that he describes the so-called optimal conditions under which his experiments were carried out. He states: "All treatments described in the preceding chapters have been made on yeast grown under optimal conditions. At the time of inoculation the yeast has been in vigorous *fermentation*, and immediately before it has been inoculated several times over on fresh wort. It has for a long period had good nutrition conditions" (p. 498). The so-called "normal" cytological pictures presented by him are those of fermenting cells and it is such fermenting cells which were exposed to the action of various chemicals. Is it surprising then that he could not arrive at any definite conclusions! It is obvious that he has not observed typical mitotic divisions in his strain and his illustrations show a remarkable similarity to those presented by Subramaniam (1948 *b*). When the same strain under different physiological conditions shows entirely dissimilar pictures (Subramaniam, 1946, 1948 *b*) of nuclear behaviour, a rational understanding of such changes is a pre-requisite for any evaluation of the results obtained after treatment with either physical or chemical agencies.

Fermenting cells are refractory to ordinary fixatives and stains and Levan's remark that they were "whimsical" is confirmed by our experience. Levan suggests that many of the factors may not be under the control of the operator. That there is no reason for such pessimism has already been expressed by Subramaniam (1948 *a*). It is from the cytological pictures observed in fermenting cultures that Levan asserts that the low chromosome numbers published for yeasts may be the result of fusions. "The chromosome number is higher than has generally been assumed, ten being a minimum number" (Levan, 1947, p. 464). Levan's observations were solely confined to fermenting cultures. It is known that fermenting cells are endopolyploid (Subramaniam, 1948 *b*). Therefore, it stands to reason that the normal chromosome number of the strain employed by him should be much lower during typical mitotic division in the aerobic phase than that assumed. An ascending grade of chromosome numbers was illustrated by Subramaniam (1948 *b*) in a five-day old fermenting culture stimulated to divide by addition of fresh medium. Levan admits that polyploid yeasts

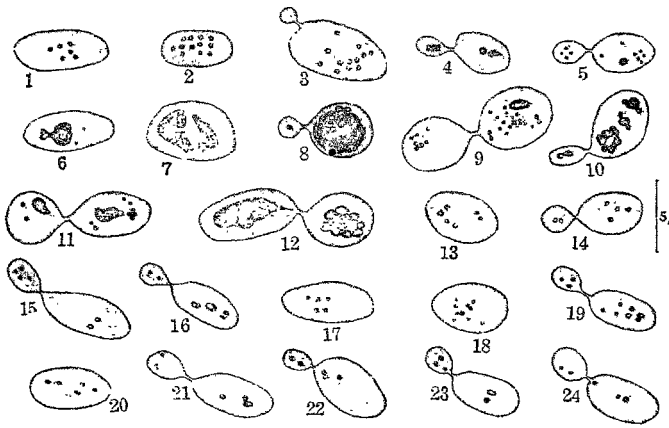
may have originated during the course of investigations by other workers If that is so, there is every likelihood that his *control strain* itself may be polyploid. It is known that even chromosome size is under genetic contro (Darlington, 1937) and hence any generalisation based on abnormalitie observed in cultures fermenting normally or in the presence of variou chemicals cannot be valid.

OBSERVATIONS

The observations described below are from the same slides from which the normal and abnormal mitotic divisions in the distillery yeast were described in the previous contribution (Ranganathan and Subramaniam 1948). The technique of handling yeasts for cytological investigations was that devised by Subramaniiam (1948 *a*). An identical procedure has been followed in illustrating and describing the various stages. It was shown earlier (Ranganathan and Subramaniam, 1948) that the chromophobic core of the chromosomes could be identified as the heterochromatin. Owing to staining and other difficulties this phenomenon was not universal. In the figures presented in this paper also, chromosomes showing chromophobic cores are illustrated with a stippled interior, while those lacking them are inked completely.

Hexaploid.—Fig. 1 shows the metaphase of the hexaploid and the anaphase of the same is illustrated in Figs. 2 and 3. Though such cells may show the origin of buds (Fig. 3), regular segregation into equal complements is very rare. If either the bud or the mother cell gets a complement of six chromosomes, usually the other group shows laggards and micronuclei formation (Fig. 4). Since such pictures are rare, one gets the impression that this may be the condition immediately following the formation of a hexaploid cell. The twelve daughter chromosomes may show an irregular distribution. Four of them may pass on to the bud and those remaining may persist in a scattered condition in the mother cell (Fig. 5). Some of these may even form micronuclei (Fig. 5).

The cells with a higher chromosome complement show amitotic phenomena and pycnosis (Figs. 6 and 7). Whether these originate by abnormal mitotic divisions of the hexaploid alone or whether they can arise from the pentaploid also is very difficult to decide. The two nuclei resulting from an amitotic division may be unequal (Fig. 8). A dividing cell with unequal complements of daughter chromosomes is illustrated in Fig. 9. This condition is probably succeeded by the formation of several nuclei which are amœboid in shape (Figs. 10 and 11). That lagging chromosomes may often get incorporated in the nuclei is suggested by the curious disposition and



FIGS. 1–24.—Fig. 1. Metaphase of hexaploid. Figs. 2 and 3. Early anaphase of the hexaploid. Fig. 4. The bud has six chromosomes, but the mother cell shows micronuclei formation. Fig. 5. The bud has four chromosomes, while the mother cell has eight, one of the latter having been transformed into a nucleus. Figs. 6 and 7. Amitosis in cells originating apparently as in Fig. 5 or in Fig. 19. Fig. 8. Pycnotic nucleus in the mother cell and a smaller one in the bud. Fig. 9. Large number of daughter chromosomes showing irregular separation as well as micronuclei formation. Fig. 10. Multinucleate cell. Figs. 11 and 12. Lagging chromosomes and micronuclei formation. Fig. 13. The six chromosomes appear in groups of two and four. Fig. 14. The smaller complement has passed on to the bud. Fig. 15. The bud has four chromosomes while the mother cell has only two. Fig. 16. Micronuclei formation in a dividing cell showing four chromosomes. Fig. 17. Metaphase of Pentaploid. Fig. 18. Early anaphase of pentaploid. Fig. 19. Irregular distribution of the daughter chromosomes. Fig. 20. Segregation of five chromosomes into groups of two and three. Fig. 21. The bud has two chromosomes while the three remaining in the mother cell show micronuclei formation. Fig. 22. The mother cell has three while the bud has two chromosomes. Fig. 23. The bud has three while the mother cell has two chromosomes. Fig. 24. Chromosome lagging.

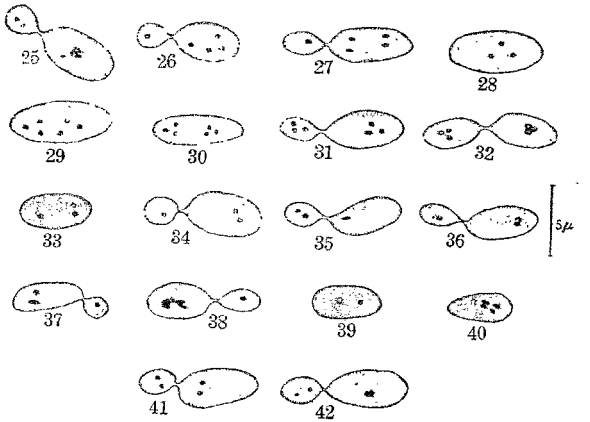
shape of the nuclei in Figs. 11 and 12. However, the staining of the cells showing amitotic phenomena and multiple nuclei formation indicated pycnosis and hence eventual death.

A perusal of Fig. 5 would suggest that the bud having four chromosomes may be viable. That there is an attempt to reconstitute the original number four, even without any division of the chromosomes is illustrated in Figs. 13, 14 and 15. The six chromosomes initially segregate into groups of four and two (Fig. 13). Either of these complements can pass on to the

bud. The daughter cell contains two chromosomes while the mother has four in Fig. 14, and a reversal of the condition is shown in Fig. 15. Even in such cells micronuclei formation can occur. The mere possession of four chromosomes does not mean that they can work in harmony. Two are forming micronuclei in Fig. 16.

Pentaploid.—The metaphase of the pentaploid has a characteristic arrangement of chromosomes (Fig. 17). These may divide to give ten chromatids (Fig. 18) but the segregation is irregular (Fig. 19). It is quite likely that some of the abnormal nuclei showing pycnosis described in connection with the hexaploid (Figs. 6, 7, 8, 9, 10, 11 and 12) may also have originated from the products of irregular segregation of the daughter chromosomes in the pentaploid. What is much more important is that like the hexaploid, the pentaploid also shows a segregation of the chromosomes without splitting into chromatids. The five chromosomes appear in two groups of two and three respectively in Fig. 20. The distribution of the complements is not rigid (Figs. 21, 22, 23 and 24). Two chromosomes may pass on to the bud (Figs. 21, 22 and 24), or the bigger complement may migrate to the bud (Fig. 23). The curious distribution illustrated in Fig. 24 suggests that the odd one may disintegrate in the cytoplasm. A slight modification of the above condition appears in Fig. 25. Two of the chromosomes in the mother cell are trying to reconstitute a nucleus leaving the odd one out. It does not appear quite necessary that the chromosomes of the pentaploid should segregate into complements of three and two. The tetraploid condition appears to be reconstituted by the elimination of a single chromosome (Figs. 26 and 27). Here only the single chromosome has been observed to migrate to the bud.

Triploid.—Since the pentaploid gives rise to triploids (Figs. 19, 20, 21, 22, 23 and 24), the behaviour of the chromosomes of the triploid assumes added importance. The three chromosomes at metaphase show a triangular arrangement (Fig. 28). The regular mitotic division which probably occurs immediately after the origin of such a type is illustrated in Figs. 29, 30, 31 and 32. The six daughter chromosomes separate into equal groups retaining their characteristic triangular arrangement and one of them passes on to the bud (Figs. 31 and 32). The chromosomes may (Figs. 28, 29, 31 and 32), or may not show a heterochromatic core. As in the case of the hexaploid and the pentaploid, in the triploids also there is segregation without chromosome division. In Fig. 33 one of the chromosomes lies separated from the other two and probably migrates later to the bud (Figs. 34 and 36). The reverse procedure is seen in Fig. 35, where the single chromosome



FIGS. 25–42.—Fig. 25. The two chromosomes in the mother cell trying to reconstitute a nucleus. The lagging chromosome is apparently left out. Figs. 26 and 27. One of the chromosomes in the pentaploid passes on to the bud. Fig. 28. Metaphase of the triploid. Figs. 29, 30, 31 and 32. Regular anaphasic separation of the daughter chromosomes with one group passing into the bud. Fig. 33. Segregation of the unsplit chromosomes into groups of two and one. Fig. 34. The bud has one while the mother cell has two chromosomes. Fig. 35. The bud has two while the remaining chromosome in the mother cell is pycnotic. Fig. 36. The chromosomes in the mother cell are trying to reconstitute a nucleus. Fig. 37. Micronuclei formation in a triploid cell which is dividing without the splitting of chromosomes. Fig. 38. Pycnotic Nucleus in the mother cell. Fig. 39. Diploid metaphase. Figs. 40, 41 and 42. Regular anaphasic separation of the daughter chromosomes in a diploid.

appears rod-shaped and intensely stained. The different sizes of the two chromosomes in the mother cell in Fig. 37 suggested a non-viable combination as evidenced by the irregular shape of the nucleus in Fig. 38.

Diploid.—The diploid shows regular mitoses (Figs. 39, 40, 41 and 42). It was emphasized that the products of division of the hexaploid, the pentaploid and the triploid having apparently viable chromosome numbers show micronuclei formation. This would suggest that particular genic constitutions alone render viable even those cells having two or four chromosomes. The rest apparently disintegrate. When cells possessing two chromosomes may or may not be viable, it appears from Figs. 39, 40, 41 and 42 that only viable combinations show normal mitosis. Since these could not be isolated by plating the original culture, the logical conclusion is that these

undergo a somatic doubling and reconstitute the original tetraploid condition. Unless such a possibility is visualised, the supernumerary divisions resulting in the formation of cells having either four or two chromosomes have no significance. Once it is assumed that the diploid becomes a tetraploid by somatic doubling after origin by mitotic aberrations either from the original tetraploid or from the various other chromosomal types, the need for such a mechanism to reconstitute the tetraploid condition appears to fit in a well ordered scheme. Indirect evidence that a gene mutation should precede any induction of tetraploidy has recently been presented (Subramaniam and Sreepathi Rao, 1950). This aspect of the question is dealt with in the discussion.

A pictographic summary of the mitotic aberrations and the fate of the various types originating as a result of such irregularities is given in Fig. 43.

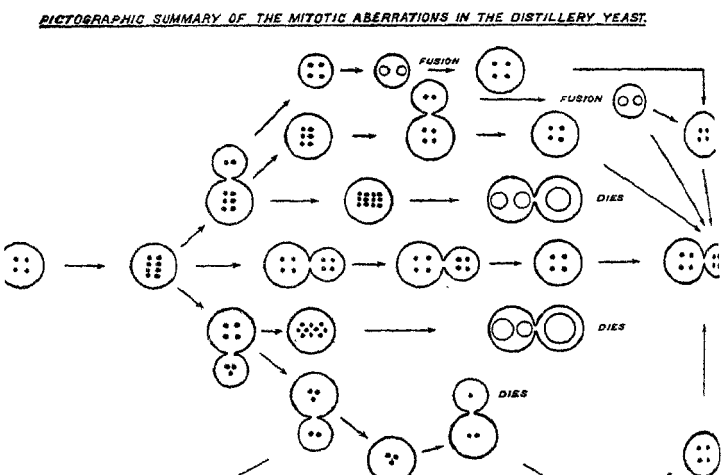


FIG. 43. Pictographic summary of the mitotic aberrations in the distillery yeast.

DISCUSSION

The yeast strains whose cytology has been the subject of a number of contributions from this laboratory have been under constant observation

for the past seven years. The results incorporated in this paper are based on more than a hundred experiments. When there are mitotic aberrations in a tetraploid, cells with differing chromosome complements have naturally to be expected. The stability of the various types is not germane to the issue. Even if some of them are not viable, as is reported here, they persist in cultures for varying periods. One has therefore, to expect in any smear (i) normal cells dividing regularly, (ii) cells segregating unbalanced chromosome complements and (iii) the products of such abnormal divisions. If the cells arranged in the pictographic summary (Fig. 43) are jumbled together, the picture may at first be confusing. But it is apparent that they are capable of being identified and arranged in a rational manner. It has to be emphasized that the peculiar cytological behaviour of the distillery yeast is nothing new. Vaarama (1949) records: "The cells which possess deviating chromosome numbers have not been concentrated on any of the different meristematic zones of the root-tip, *i.e.*, periblem, plerome or dermatogen. Neither has it been observed that any large cell groups consist of cells provided with a certain chromosome number". He adds: "A pertinent phenomenon visible in all root-tip meristems studied is the occurrence of numerous dead and degenerated cells among the living cells. This suggests that a certain part of the cells, probably among those that have obtained the decreased chromosome number, have not been viable" (p. 144). How reminiscent this is of the phenomena discussed in this paper needs no reiteration. It should be obvious that the cytology of yeasts is in no way different from that of higher organisms.

It was concluded in the earlier contribution (Ranganathan and Subramaniam, 1948) that the cause for the mitotic aberrations may be the result of an accentuation of the somatic pairing of the chromosomes due to autopolyploidy. The aberrations were explained on the basis of a precocious resumption of the pairing force between sister chromosomes or groups of sister chromosomes at the commencement of the separation into daughter groups. The differences in the pairing force between groups of daughter chromosomes was suspected to be the result of the mutant genes in some of the chromosomes. Mutations in the diploids and the induced autotetraploids need not be in identical directions. It is known that polyploids usually lose their initial gigantism by recombinations, gene mutations or even losses of parts by chromosomes. If that is so, the auto-tetraploid distillery yeast should have undergone considerable selection enabling it to survive. During this process, the two pairs of chromosomes may have undergone a differentiation and may not have identical gene sequences. That any two or four of them cannot reconstitute a viable combination

would have become evident from Figs. 13, 16, 23 and 37, which show micronuclei formation. The duplicated chromosome complements are virtually becoming separated into differentiated pairs. Only when a cell receives a pair of chromosomes having almost identical gene sequences does it appear to be viable.

It was suspected earlier (Subramaniam, 1947) that the abnormal mitoses as well as the behaviour of the products having unbalanced chromosome numbers may be the result of nucleic acid upsets (Darlington and Thomas, 1941). That explanation had to be given up because a simpler genetic interpretation based on irregularities in the somatic pairing force was possible (Ranganathan and Subramaniam, 1948). The so-called sex-ratio condition in *D. pseudo-obscura* (Sturtevant and Dobzhansky, 1936; Dobzhansky, 1937) is found in as many as 30 per cent. of the individuals in some populations. When that curious condition is suggested to be the result of " position effects " (White, 1945) a similar explanation for the occurrence of abnormal mitoses leading to the formation of cells deviating from the normal as regards their chromosome number appears to be highly probable. Vaarama (1949) ascribes the origin of cells having varying chromosome numbers in a colchicine-induced tetraploid *Ribes nigrum* to spindle abnormalities. In the distillery yeast the condition reminiscent of that in *Ribes* appears to be the result of entirely different factors. When spindle abnormalities themselves are considered to be under genic control like many other steps in mitotic division, the mitotic aberrations in the distillery yeast should themselves be determined by genetic factors.

The hexaploid, the pentaploid and the triploid are unable to multiply normally by mitosis. But as would have become evident from the observations illustrated, they exhibit mechanisms by which attempts are made to regain the original tetraploid constitution. Supernumerary divisions immediately after meiosis with (Darlington and Thomas, 1941) or without the division of the chromosomes (Beadle, 1931) have been classified under a separate category called polymitosis. Whether the division of the yeast cells having unbalanced chromosome numbers without any splitting of the chromosomes could be included under this category is questionable. They have a remarkable similarity, but the comparison between the observations recorded in this paper and the supernumerary divisions observed in *Zea* and *Sorghum* cannot be extended further.

Darlington and Thomas (1941) remark: " In every instance we therefore have reason to associate nuclear polymitosis with nucleic acid irregularity " (p. 48). That this generalisation may not be valid would become

apparent from the published literature. In scale insects, Hughes-Schrader (1935) and Schrader (1929) have described some peculiar meiotic phenomena. In *Phenacoccus acericola* (Hughes-Schrader, 1935) a haploid set of chromosomes is heterochromatic even in the somatic cells of the blastula. During the prophase of the first meiotic division, these show precocious nucleination and at the second division again they are heteropycnotic. The unipolar or "half spindle" separates the heteropycnotic chromosomes from the others which are flocculent and appear lightly stained. It is the latter which give origin to the sperms, the heteropycnotic groups being cast off with a mass of cytoplasm. What has to be emphasized is that (*a*) haploid groups show differences in their nucleic acid charge and (*b*) the unipolar spindle separates from the rest, the heteropycnotic group destined to disintegrate. This does not appear to be an isolated instance (White, 1945). These observations indicate that heteropycnosis and spindle abnormalities are themselves genetically controlled.

Division of the cell without any formation of daughter chromosomes appears to be a mechanism to enable even lethal chromosomal combinations to produce the original chromosome constitution. The hexaploid produces a diploid as well as a tetraploid, both or either of which may be non-viable. The pentaploid gives rise to the triploid and diploid, or a tetraploid and a haploid. The triploid again shows division into a diploid and a haploid. That the mechanism is not perfect would be evident from the formation of micronuclei in cells showing chromosome numbers which apparently ought to be viable. The progressive reduction in the number of chromosomes results finally either in the formation of a tetraploid or a diploid. The other types have either to produce the above viable combinations or perish. In this connection it is interesting to remember that a similar mechanism may be operating even in highly endopolyploid fermenting yeast cells stimulated to divide by addition of fresh wort. Subramaniam (1948 *b*) illustrated in his Figs. 22 and 23, buds having two chromosomes. He raised the question (p. 329) whether such unequal segregation resulting in the formation of a bud with two chromosomes may be a method by which a diploid cell takes its origin from an endopolyploid cell. It was definitely stated by him that no method was available by which such buds with two chromosomes could be isolated and studied. That was in fermenting cells of the two chromosome brewery yeast (Subramaniam, 1946, 1948 *b*; Prahlada Rao and Subramaniam, 1952). When similar phenomena leading to the formation of individuals with the normal chromosome number occur in the distillery yeast having apparently lethal chromosome complements, such a probability is highly suggestive. We are probably seeing under

entirely different physiological conditions two different aspects of the same mechanism enabling cells with abnormal complements to attempt a reconstitution of their original chromosomal constitution.

The selective elimination of some of the chromosomes during the formation of the male and female somatic cells in *Sciara* (Metz, 1939; White, 1945) indicates that here a different mechanism is in operation. The attempt by those yeast cells having unbalanced chromosome numbers to reconstitute the original four chromosome condition by irregular segregation of the unsplit chromosomes seems to be a regulating and stabilising mechanism developed to compensate the lethal effects of the mitotic abnormalities.

The diploid shows regular anaphase separation of its chromosomes (Figs. 40, 41 and 42). But the fact that it could not be isolated by plating indicated that it should have undergone a "somatic doubling" at a very early stage after origin. Evidence for such a possibility is available from an entirely different direction. The naturally occurring tetraploid distillery yeast (Ranganathan and Subramaniam, 1948) and the auto-tetraploid brewery yeast isolated after acenaphthene treatment (Subramaniam, 1945, 1947) are sporogenous. Their spores should have the diploid chromosome complements. Some of the asci in both strains contain but a single spore and because of their balanced chromosome constitution should be capable of unlimited vegetative proliferation without fusion with other spores. And yet, during the past four years, no diploid has been isolated either from the distillery or the brewery tetraploid cultures. It was logical under the circumstances to surmise that when a spore does not fuse with another, it undergoes a somatic doubling to reconstitute the vegetative chromosome number. Because the spore has a balanced diploid chromosome complement, the necessity for a somatic doubling even in such spores should be the result of some change synchronising with or preceding the origin of the tetraploid itself. If induction of auto-tetraploidy consists merely in a doubling of the diploid chromosome complement, then the condition cannot be stable since isolated spores as well as diploid cells arising by mitotic aberrations should be capable of normal proliferation. In other words, the periodic appearance of diploids in auto-tetraploid cultures should be a common phenomenon. Since the observations recorded in this paper as well as those on the auto-tetraploid brewery yeast (Duraiswami and Subramaniam, 1950; Ranganathan and Subramaniam, 1950) indicate an absence of origin and persistence of diploids in cultures of autotetraploids, it appeared that even those diploids which arise accidentally become tetraploid by somatic doubling of the chromosome complement. It has to be

distinctly remembered that mere inhibition of cell division would not result in the production of a tetraploid. The tetraploid chromosome complement should be capable of functioning harmoniously. From experiments on the effect of camphor on yeasts Subramaniam and Sreepathi Rao (1950, 1952) emphasized that a gene mutation should precede any induction of tetraploidy and that colchicine and acenaphthene may differ from the other so-called C-mitotic substances in that they are capable of producing a gene mutation at a specific locus. Since sporadic but spontaneous origin of tetraploids have been observed in our two chromosome brewery yeast (Prema Bai and Subramaniam, 1947; Subramaniam and Krishna Murthy, 1949) it appeared that the mutations at the "polyploid" locus should occur at a relatively low frequency. If tetraploidy is itself the result of a gene mutation, the same mutant allele in an isolated spore, or a diploid vegetative cell originating from the tetraploid, should produce a "somatic doubling". That is what apparently happens in the diploid originating from the original tetraploid distillery yeast or from the hexaploid, pentaploid and the triploid. The fact that the diploid transforms itself into a tetraploid by a spontaneous doubling of the chromosome set gives added confirmation to the belief that the distillery yeast under consideration is an auto-tetraploid.

The question naturally arises whether the cytological observations recorded above have any significance in the evaluation of the genetic behaviour of yeasts. It would be obvious that even single cell or single spore isolations would not give a "pure" culture. A culture which has been grown from an isolated cell having a specific genic complex would during vegetative division become a mixed one owing to the formation of new types by irregular segregation of the unsplit chromosomes and by "somatic doubling". When investigators on genetics of yeasts (Winge and Roberts, 1948; Lindegren and Lindegren, 1946) have not even taken into consideration the probability that the strains employed by them may be polyploids (Subramaniam, 1950 *a*, *b*, *c*) and when the "purity" of gametes is meaningless in polyploids, the peculiar cytological behaviour of the auto-tetraploid distillery yeast emphasizes the need for caution in offering radical theories to explain some curious segregations observed in hybridization experiments.

References

1. Beadle, G. W. .. "A gene in maize for supernumerary cell divisions following meiosis," *Cornell Univ. Expt. Sta. Ithaca*, 1931, *Mem.* 135, 1–12.

2. Darlington, C. D. .. *Recent Advances in Cytology*, 1937. J & A. Churchill, London.

3. Darlington, C. D. and Thomas, P. T. — "Morbid mitosis and the activity of inert chromosomes in *Sorghum*," *Proc. Roy. Soc. Lond.*, 1941, **130 B**, 127–51.
4. Dobzhansky, Th. .. *Genetics and the Origin of Species*, 1947, Columbia University Press.
5. Duraiswami, S. and Subramaniam, M. K. — "Reversal of some chromosomal mutations in yeasts," *Cellule*, 1950, **53**, 215–55.
6. ———— .. "Further Evidence for Mitosis in Yeasts," *Experientia*, 1951, **7**, 422.
7. Guilliermond, A. .. *The Yeasts*, John Wiley & Sons, Inc., New York, 1920.
8. Hughes-Schrader, S. .. "The chromosome cycle of Phenacoccus (coccidæ)", *Biol. Bull. Woods Hole*, 1935, **69**, 462–68.
9. Levan, A. .. "Studies on Camphor Reaction of Yeast," *Hereditas*, 1947, **33**, 457–514.
10. Lindegren, C. C. and Lindegren, G. — "The cytogene theory", *Cold Spring Harb. Symp. Quant. Biol.*, 1946, **11**, 115–29.
11. Metz, C. W. .. "Observations on evolutionary changes in the chromosomes of Sciara (Diptera)", *Publ. Carneg. Inst. Washington*, 1938, **501**, 274–94.
12. Prahlada Rao, L. S. and Subramaniam, M. K. — "Somatic chromosomal reduction in yeast," *Archiv fur Mikrobiologie*, 1952 *a*, **17**, 160–165.
13. ———— .. "Studies on the Cytology of Yeasts. VII. Nuclear phenomena in cells from 24-hour Agar Slants," *Proc. Ind. Acad. Sci.*, 1952 *b* (In press).
14. Prema Bai, M. and Subramaniam, M. K. — "Rate of growth of Diploid and Tetraploid Yeasts," *Curr. Sci.*, 1947, **16**, 380–81.
15. Ranganathan, B. and Subramaniam, M. K. — "Centrosomes in Yeasts," *Sci. & Cult.*, 1947, **12**, 478–81.
16. ———— .. "Studies on the Cytology of Yeasts. V. Normal and Abnormal Mitoses in a Distillery Yeast," *Proc. Nat. Inst. Sci., India*, 1948, **14**, 389–404.
17. ———— .. "Studies on the Mutagenic Action of Chemical and Physical Agencies on Yeasts. I. Induction of Polyploidy by Diverse Agencies," *J. Ind. Inst. Sci.*, 1950, **32 A**, 51–72.
18. Schrader, F. .. "Experimental and cytological investigations of the life cycle of *Gossyparia spuria* (coccidæ) and their bearing on the problem of haploidy in the males," *Zeitschr. Wiss. Zool.*, 1929, **134**, 149–79.
19. Skovsted, A. .. "Induced camphor Mutations in Yeast", *C. R. Lab. Carlsberg. Ser. Physiol.*, 1948, **24**, 249–62.
20. Sturtevant, A. H. and Th. Dobzhansky — "Geographical distribution and Cytology of 'sex-ratio' in *Drosophila psuedoobscura* and related species," *Genetics*, 1936, **21**, 473–90.
21. Subramaniam, M. K. .. "Induction of Polyploidy in *Saccharomyces cerevisiæ*," *Curr. Sci.*, 1945, **14**, 234.
22. ———— .. "Studies on the Cytology of Yeasts. I. Mitosis in *Saccharomyces cerevisiæ*," *Proc. Nat. Inst. Sci. India*, 1946, **12**, 143–49.

23. Subramaniam, M. K. .. "Studies on the Cytology of Yeasts. II. Induction of Polyploidy and heterochromatin," *ibid.*, 1947, **13**, 129–39.

24. ——— .. "Studies on the Cytology of Yeasts. III. The technique of handling yeasts for cytological investigations," *ibid.*, 1948 *a*, **14**, 315–323.

25. ——— .. "Studies on the Cytology of Yeasts. IV. Endopolyploidy in Yeasts," *ibid.*, 1948 *b*, **14**, 325–33.

26. ——— .. "The Problem of Haploidy in Yeasts," *J. Ind. Inst. Sci.*, 1950 *a*, **32 A**, 29–40.

27. ——— .. "Haploidy and the Species Concept in Yeasts," *ibid.*, 1950 *b*, **32 A**, 41–50.

28. ——— .. "A Critical Evaluation of the Question of Cytoplasmic Inheritance in Yeasts," *ibid.*, 1950 *c*, **32 A**, 73–90.

29. ——— .. "Chromosomes in Yeasts," *Nature*, 1951 *a*, **168**, 427.

30. ——— .. "Tetraploidy in Yeasts," *Cellule*, 1951 *b*, **54**, 145–48.

31. ——— .. "On the Identification of the Various Cell Structures in Yeast," *J. Ind. Inst. Sci.*, 1952, **34 A**, 11–23.

32. —— and Ranganathan, B. "Peculiar cytological behaviour of a distillery yeast," *Nature*, 1946 *a*, **157**, 50.

33. ——— .. "Staining the chromosomes of yeast by the Feulgen technique," *Nature*, 1946 *b* **157**, 657.

34. ——— .. "Chromosome constitution and characteristics of giant colonies in yeasts," *Proc. Nat. Inst. Sci. India*, 1948, **14**, 279–83.

35. —— and Krishna Murthy, S. N. "Effect of Acenaphthene on Yeast Strains of Different Genic and Chromosomal Constitution," *Proc. Ind. Acad. Sci.*, 1949, **30 B**, 185–94.

36. —— and Sreepathi Rao, S. K. "Gene Mutations induced by Camphor in Yeasts," *Research*, Lond., 1950, 3, 49–50.

37. ——— .. "Studies on the Mutagenic Action of Chemical and Physical Agencies on Yeasts. IV. The Effect of Camphor on Fermenting Yeast Cultures," *Proc. Ind. Acad. Sci.*, 1952, **35 B**, 1–27.

38. Vaarama, A. .. "Spindle abnormalities and variation in chromosome number in *Ribes nigrum*," *Hereditas*, 1949, **35**, 136–62.

39. White, M. J. D. .. "Animal Cytology and Evolution," Cambridge University Press, 1945.

40. Winge, O. and Roberts, C. "Inheritance of enzymatic characters and the phenomenon of Long-Term adaptation," *C. R. Lab. Carlsberg. Ser. Physiol.*, 1948, **24**, 263–315.