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Report Number: ORNL- 696
Health and Biology
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SEYMOUR POMPER

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Recent Developments in Yeast Genetics

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Yeast has become an increasingly important genetic subject. Ease of culture, speed of growth, well-characterized physiology, and industrial significance have been contributing factors in the developing interest in yeast genetics. This review will limit itself, in the main, to a consideration of the work that has been done in yeast genetics in the past decade.

Much of the earlier knowledge of yeast life cycles and genetics has been summarized by Guilliermond (1, 2) and Henrici (3). Guilliermond (4, 5, 6), at the beginning of the twentieth century, showed that isogamic copulation preceded ascus formation in three species of Schizosaccharomyces, viz., S. octosporus Beijerinck, S. pombe Lindner, and S. mellacei Jorgensen. In these papers, conjugation and spore formation were described in some detail. Guilliermond observed that two vegetative cells functioned as gametes, fusing by means of a connecting canal put out by each cell, to form a large oval zygote. Sometimes the gametes did not fuse completely, and the resulting zygote consisted of a cell swollen at either end into which the dividing nuclei migrated at meiosis. In 1901, Barker (7) also observed the latter type of conjugation process in a Zygosaccharomyces yeast. Guilliermond (1, 8, 9) later reported heterogamic conjugation in a number of yeasts. He described the mechanism as follows (2): "One is a big adult cell assuming the part of a female gamete, the other is a young and not fully developed bud acting as a male gamete. Both unite through a canal, the male gamete discharging all its contents into the female which then develops into an ascus." In 1926, Nadson and Konokotine (10) described heterogamic fusion in

the genus Nadsonia. In this yeast, the "female" gamete, having received the contents of the "male", buds off its whole contents. The two nuclei fuse within the bud-cell, which then differentiates into an ascus with 1-4 spores.

Although the view is no longer held that "sex", in the sense of two differentiated gametes conjugating, is the rule in the yeasts, these early observations have much merit. Two life cycles, "sexual" in the sense of alternation of generations due to the fusion of two cells having gametic function, were clearly described: the Zygosaccharomyces type and the Nadsonia type. In both of these yeasts, the diploid stage is usually transient, being maintained only for the period immediately preceding reduction division. Spore formation in the genus Saccharomyces, on the other hand, was considered to be largely through parthenogenesis, since conjugation was not observed.

Early Genetic Studies with Saccharomyces

A marked step forward was taken when Kruis and Satava (11) and Winge (12) independently observed alternation of generations in Saccharomyces. Winge (12), in 1935, recognized the existence of a diplophase and a haplophase in S. cerevisiae Hansen, and showed that the vegetative cells of this yeast are diploid, produced by conjugation of two spores or gametes derived from spores. These observations suggest (3) that few, if any, of the spore-forming yeasts are entirely parthenogenetic with regard to spore formation as had been thought earlier (1). Winge (12) concluded, "A striking feature is the absence of an established system governing the zygote formation. Sometimes the spores unite to form a zygote before or after they have germinated singly, sometimes a spore unites with a haploid vegetative cell; or two vegetative cells conjugate, and they may be sister-cells, or mother-cell and daughter-cell, or more distantly related cells." These observations permitted the division of the sporogenous yeasts into two large classes: (1) that

in which vegetative cells are haploid with the diploid stage extending only through the short period following nuclear fusion, after which meiosis occurs and the haploid state is resumed (Zygosaccharomyces, Schizosaccharomyces, Nadsonia), and (2) that in which vegetative cells are diploid with production of haploid spores through meiosis, and subsequent fusion of two haploid cells to reproduce the diploid condition (Saccharomyces, Saccharomycodes).

Winge and Laustsen (13), in 1937, developed a micromanipulative technique by means of which the four spores of an ascus could be separated and studied. They observed that in a strain of S. cerevisiae, derived from a single cell of a culture used in pressed yeast manufacture, a single spore might give rise to a pure haploid culture, or in other cases, that the cells derived from the single spore were diploid from the beginning. They explained the latter cases by assuming that an intracellular nuclear fusion takes place within the germinating ascospore immediately following the first mitotic division. Winge and Laustsen came to the conclusion that the small globular yeasts encountered in nature were probably haploid stages of the perfect life cycle which had become established, either as a sporogenous type (Zygosaccharomyces) or an asporogenous type (Torulopsis). This inference has been questioned (14, 15) on the basis of physiological studies in which Torulopsis and Zygosaccharomyces yeasts show a wider range of ability to utilize amino acids as nitrogen and carbon sources than do Saccharomyces yeasts. It was felt that this would be unlikely if Torulopsis and Zygosaccharomyces had been derived from Saccharomyces in the recent past.

Winge and Laustsen (16, 17) carried out hybridizations of various Saccharomyces yeasts. The criteria used to characterize the crosses were primarily giant colony formation on gelatin plates, and secondarily certain biochemical characters, such as the ability to ferment sucrose, raffinose, and melibiose. They concluded that the ability to ferment a given sugar is dominant over the inability to do so.

Further, they observed that all of the yeasts used in hybridization were homozygotic with respect to fermentative ability, but were heterozygotic with respect to appearance of giant colonies.

These authors (18) in 1939 reported on genetic studies with Saccharomycodes ludwigii Hansen, a yeast in which the four ascospores fuse pairwise in germination. When the four spores of the ascus are isolated and forced to develop individually, two of the four produce normal haploid growth while the other two germinate with an elongated mycelial growth which ceases after one or two generations. The two normally germinating spores produce either both long- or both short-cell-type of growth. "It is here a matter of Mendelian segregation," Winge and Laustsen state, "as two pairs of genes called N-n and L-l produced the above mentioned types of germination. The gene N produces normal germination, whereas the gene n causes the growth to cease when a mycelial short hypha is formed. The gene L produces long-cell growth; l, short-cell growth. The vegetative cells in S. ludwigii are diploid and doubly heterozygous of the formula NnLl. Consequently all asci have this formula, too." The two adjacent spores that fuse are always complementary; if one is Nl, the other is nL, and vice versa. These results are explained by Lindegren (19) and by Winge (20) as indicating (a) that N and L are located in different chromosome pairs, (b) that during the first meiotic division L is always separated from l, and N from n, (c) that both pairs of genes are situated so close to the centromere that no crossing over between this and the genes takes place, and (d) that at the time of the second division, the nuclear spindles arrange themselves parallel to the long axis of the cell so that both nuclei give off a daughter nucleus to each end of the ascus. These findings are of considerable importance because they represent possibly the first report of Mendelian segregation in yeast (1939). The homothallic nature of the yeasts that Winge and Laustsen had been studying (13, 16, 17) had prevented the demonstration of regular Mendelian

segregation. The enforced heterothallism of S. ludwigii permitted such an analysis for the first time.

Mating Types, Crossing Procedures, Sporulation

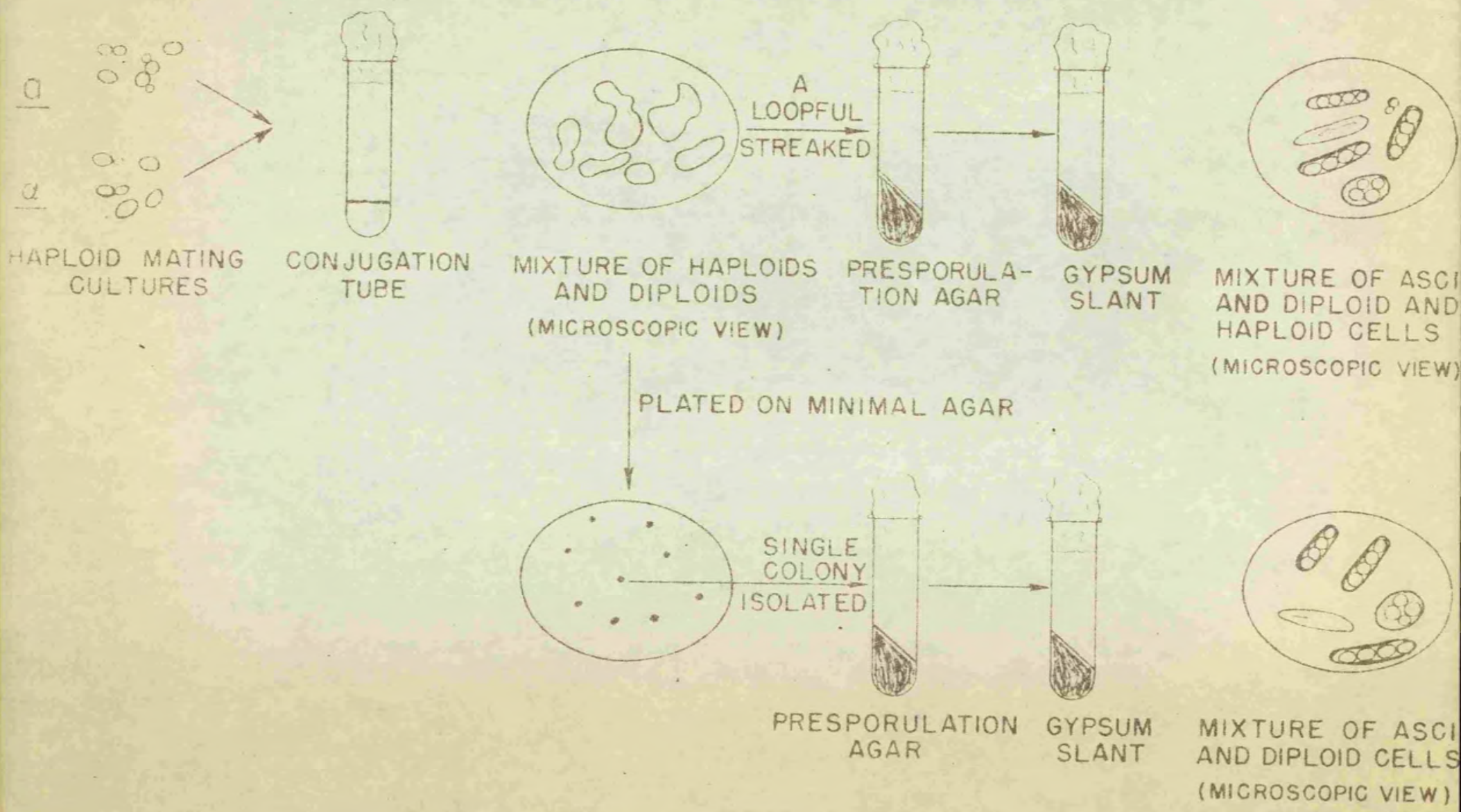
It is apparent that unless a heterothallic condition exists, it is rather difficult to establish breeding programs and follow the segregations of genes in an organism. Hence, the report by Lindegren and Lindegren (21) in 1943, that "monohybrid allelism modified by other factors controls copulation" is of fundamental importance. They assigned the symbols "a/α" to designate the mating type alleles. Their observation of heterothallism has been the basis of a great deal of genetic work with haploid strains which could be propagated vegetatively, or put through a sexual cross, at will.

The initial observation by the Lindegrens that there are two mating types in at least some Saccharomyces species has been confirmed (22, 23). Lindegren devised a crossing procedure (24) which may simply be described as "mass mating". Large numbers of cells of opposite mating type were placed together in nutrient broth, and numerous conjugations could be observed within 24 hours. The resultant mixture of haploids and diploids were then induced to sporulate (25) and the four-spored asci selected for genetic analysis. Winge and Roberts (22) have objected to this procedure on the grounds that selfing (a x a, or α x α) may sometimes occur and give rise to asci at sporulation. These authors (22) point out that the "illegitimate diploids", which Lindegren has characterized (19) as not giving rise to viable four-spored asci, may sometimes do so. Hence, they suggested that the only reliable crossing procedure was one in which two gametic cells were placed together in a hanging drop, and conjugation between them observed directly under the microscope (16). Lindegren (26) acknowledged that this criticism might be justified under certain conditions, but points out that in crosses involving

numerous genetic markers, it is a simple thing to demonstrate heterozygosity. It is proper to note, however, that Lindegren's technique may still be questioned, since in his sporulation procedure, he uses a mixed population, i.e., asci arising from many conjugations. Thus, if gene y mutated to Y, in a single cell of one of the haploids, some of the diploids would receive y, and others Y, from this potential conjugant or its progeny. Then the asci formed from these diploids would give "aberrant" results for one gene, even though heterozygosity was demonstrable by means of other genes (markers). Since a mass mating procedure has numerous obvious advantages over the laborious micromanipulative procedure in terms of speed and simplicity, the following procedure has been suggested (23) as a means of obtaining a diploid population of homogeneous genetic background. Haploids of opposite mating type, having complementary nutritional requirements, are crossed by the Lindegren procedure (24) and heterozygous diploids recovered by isolation of single colonies from minimal agar lacking all of the factors that the haploids required. Thus, for example, in a cross of a haploid which required the amino acids methionine and tryptophan, by another haploid of opposite mating type that required the nucleic acid components adenine and uracil, only the heterozygous diploid arising from the cross could grow on an agar lacking all four compounds. By this procedure, the isolated diploid colony is the source of genetic material (ascospores). Figure 1 illustrates in outline form the original and the modified procedures. This technique has certain other valuable features. It can be used to recover diploids even in those cases where conjugation between the haploids has occurred only with great rarity, and so can be used to test the mating type where it would be very difficult to do so by microscopic observation. Dominance relations are also indicated by this procedure, since the phenotype of the diploid will be determined by the dominant genes. As will be indicated later, the question of whether two phenotypically identical mutants are genotypically identical (allelic) can be answered

Figure 1

Schematic representation of the mass mating procedure of Lindegren and the modification of Pomper and Burkholder. Note that in the original procedure a mixture of diploids arising from numerous individual conjugations is carried through the sporulation procedure, and are the source of genetic material. In the modified procedure, the asci are formed from a single, isolated diploid which arose as the result of a single conjugation.


a
α
HAPLOID MATING CULTURES
CONJUGATION TUBE
MIXTURE OF HAPLOIDS AND DIPLOIDS
(MICROSCOPIC VIEW)
A LOOPFUL STREAKED
PRESPORULATION AGAR
GYPSUM SLANT
MIXTURE OF ASCI AND DIPLOID AND HAPLOID CELLS
(MICROSCOPIC VIEW)
PLATED ON MINIMAL AGAR
SINGLE COLONY ISOLATED
PRESPORULATION AGAR
GYPSUM SLANT
MIXTURE OF ASCI AND DIPLOID CELLS
(MICROSCOPIC VIEW)

very simply in this way. Two potential sources of error still remaining are the possible occurrence of mutation either in the diplophase or after segregation, and these possibilities must be recognized. In the large majority of cases, however, the results obtained with this technique have been very regular, and amenable to analysis (23).

Winge and Roberts (27) have recently (1949) encountered another interesting phenomenon with regard to mating characteristics. In a cross of a heterothallic S. cerevisiae by a homothallic S. chevalieri, an apparently tetrapolar mating scheme was encountered. This was finally explained on the basis of a gene controlling selfing, which was obscuring the bipolar mating reaction. According to their analysis, the diploid S. chevalieri is homozygous for this "diploidization" gene, and S. cerevisiae is homozygous for the recessive allelomorph. Thus, in a cross of these two, it was to be expected that two spores in each ascus would self, and two would not. The two haploid cultures which do not self are capable of mating with haploids of the opposite mating type.

Once a cross has been carried out and a diploid recovered (23), it is necessary to induce sporulation and meiosis. At meiosis, the diploid genome separates into four products (the ascospores), each of which has the genetic characteristics of a single chromatid from a given tetrad of chromatids. This is one of the reasons that yeast is good genetic material. The ability to recover the four products of meiosis and to carry out "tetrad analysis" permits an exact measure of the genetic segregation. This is clearly an advantage over genetic material where only the diploids of the following generation can be studied.

Sporulation of yeasts can be induced in a variety of ways. Phaff and Mrak (28, 29) have recently written an outstanding review of this subject in this journal, and very little can be added to what they have said. Adams (30) has recently reported very considerable success with a modification of the procedures

of Stantial (31, 32). Stantial found that spore formation could be induced by suspending washed cells in solutions of sugars and acetate. Adams has simplified the procedure by using solid medium and eliminating the washing and counting procedures that Stantial had found necessary. In our hands (33), the Stantial procedure gave rather variable results, but a procedure of this type has a great advantage over procedures involving complex natural media, since the nutritional environment can be exactly controlled. This might be of considerable importance for example, in experiments on the inheritance of adaptive enzymes, where the experimenter might wish to control rigidly the substrates to which the yeast was exposed during sporulation. The sporulation procedure of Lindegren and Lindegren (25) has proved satisfactory in our laboratory with the strain of S. cerevisiae tested. It is of interest to note that with this strain the morphology of the asci was quite different, depending on whether the sporulation had been carried out by the Lindegren method or the Stantial method. In the former, the asci are of two types, elongated cylindrical, and round to oval, while in the latter, only the round, oval asci are observed.

Genetic "Markers"

By a genetic "marker" is meant a unique characteristic which can be demonstrated to be heritable. Ideally (for simple genetic analysis) it should be inherited as though controlled by a single gene. In material like maize or Drosophila, it is fairly easy to use simple morphological markers since the tissue is well-differentiated. However, with yeast, and microörganisms in general, it is rather more difficult to use characteristics of morphology as markers. Therefore, it is necessary to search a little further with these organisms to find suitable heritable characteristics. Yeasts have for many years been differentiated taxonomically by their ability to ferment various carbohydrates. Hence, it was logical to try to

use the fermentative characteristics of yeasts as genetic markers. Winge and Laustsen (17), as early as 1939, used raffinose, sucrose and melibiose as test sugars in studying segregations. Lindegren and co-workers have used such characters in many crosses, and have presented extensive data on their inheritance (25, 34, 35, 36, 37, 38, 39). However, there is still some disagreement as to the genetics of the inheritance of these characters. Only a brief survey of this problem will be attempted here.

In 1944, Lindegren, Spiegelman, and Lindegren (34) reported that the ability to ferment melibiose was controlled by two pairs of dominant genes in Saccharomyces carlsbergensis, and that these genes segregated in a regular Mendelian fashion. Spiegelman, Lindegren, and Lindegren (35) later found that once synthesis of melibiozymase had been initiated, interaction between the cytoplasm and the substrate could maintain the enzyme indefinitely in the cytoplasm in the absence of the specific gene for melibiozymase production. Based on these and similar observations, the cytogene (19, 36, 40) and the plasmagene (41, 42, 43) theories were evolved to account for the apparent cytoplasm-substrate effect. Lindegren and Lindegren (26, 38, 44, 45) have since modified their views on the mode of inheritance of adaptive enzymes, and feel that the evidence no longer supports the theory of self-duplicating cytoplasmic units. Their current interpretation of ratios deviating from the 1:1 (expected for regular segregation of a single gene pair) is on the basis of transfer of genetic material from gene to gene in the heterozygote, and depletion mutations in the vegetative haploid (38, 44, 45).

Before going into further detail on the data upon which these theories have been based, it might be appropriate to introduce the views of Winge and his group on the subject of the inheritance of fermenting capacity. Winge and Roberts (22) in 1948 reported a series of experiments on enzymes, "adaptive" for galactose and maltose fermentation. They concluded that galactose fermentation was controlled

by a pair of genes, designated "G and g_s". If a culture has the "G" gene, it will be a rapid fermenter of galactose; if it has the "g_s" gene, it will be a slow fermenter ("long-term adaptation"). They interpreted their findings on the inheritance of maltose fermentation on the basis of three polymeric genes, "M_1, M_2 and M_3". Their final conclusions were that the phenomena observed by the Lindegrens (36) were explicable on the basis of "long-term adaptation" and multiple genes. In recent publications (46, 47), Winge has reiterated this view, and reports results (47) on a study of the inheritance of sucrose fermentation in a yeast, S. chevalieri. He interprets his data on the basis of three genes controlling the ability to ferment sucrose. At lease one of the three recessive genes (in an organism homozygous recessive for all three genes) controls a slow synthesis of enzyme -- analogous to "g_s" or "M_3". Winge (47) further states that S. cerevisiae appears to have two polymeric genes for the splitting of sucrose.

Mundkur (48) and Mundkur and Lindegren (49) have objected to the interpretations which Winge and Roberts (22) have placed on their results with galactose-fermentation inheritance. They report an experiment in which it could be demonstrated that a small, variable number of colonies appeared on galactose agar when glucose-grown cells of a "g_s" haploid were plated thereon. They conclude from this that the phenomenon characterized by Winge and Roberts as "long-term adaptation", viz., slow appearance of growth and fermentation, is really a process of mutation and selection. These authors would differentiate between irregular ratios observed after slow adaptation, and irregular ratios obtained when all the ascospores are characterized after only a few days. Thus "long-term adaptation" would be interpreted as mutation and selection, and more rapidly appearing irregularities would be explained on the basis of "conversion" (38).

We might here consider briefly the experimental data upon which the "conversion" and the "depletion mutation" theories are based. In 1947, Lindegren and Lindegren (44) reported on crosses involving a pink, adenine-requiring mutant (50). The pink

pigment appeared to be associated with the requirement for adenine. In a mating of the pink adenine-requiring haploid by a white adenine-independent haploid of opposite mating type, regular 2:2 segregations were observed in the majority of cases. In a few asci, aberrant ratios were found for the pigment and for the nutritional requirement. Some of the aberrant ascospores were white and adenine-dependent. When these were crossed to pink, adenine-dependent haploids of opposite mating type, four pink, adenine-dependent progeny were recovered in each of 21 asci. The explanation presented by the authors is that the white, adenine-dependent haploid parent had lost some factor in the course of vegetative propagation which is restored by the "normal" pink yeast in the cross. They suggest that this type of "depletion mutation" may occur frequently, but cannot be detected in organisms which are taken through a cross before analysis. The "conversion" hypothesis (26, 38, 39) has been invoked to explain aberrant ratios obtained in studies on the inheritance of fermenting ability towards various sugars. These sugars include galactose, melibiose, maltose, sucrose and α-methyl glucoside. The basic observation has been the occurrence of aberrant ratios when certain "converter" stocks are used. It would not be appropriate at this time to attempt a detailed analysis of these experiments. The essential feature has been that in crosses involving certain cultures (all derived from a single ascospore of S. carlsbergensis) very considerable irregularities in the segregations of a number of genes have been observed. The two characters that were most extensively investigated were the fermentation of galactose and melibiose. If the converter stock had the dominant allele, they observed (38, 39) an excess of dominant types in the segregation, and conversely, a recessive "converter" produced an excess of recessive types. It is this writer's feeling that before these new theories can be accepted as wholly substantiated, the experimental observations will have to be repeated with special emphasis on population analyses at every point in the handling of the experimental material. Since such analyses

have been run in only a limited number of cases (39, 49), it is difficult to evaluate the whole body of experiments based on unanalyzed original populations. The possibility thus has to be ruled out that aberrant ratios are due, in part at least, to unexpected early mutation, and that mixed populations with perhaps differing selective mating values have been used. As an example of how such mechanisms may cause confusion, some recent work (Pomper and Meyer, unpublished) will be cited. In a cross of a certain adenine-requiring mutant (not the same as the one used by Lindegren) by a haploid of opposite mating type, four out of the seven complete asci dissected showed aberrant ratios for adenine. The other five loci in the cross segregated regularly. It was possible to show by population analyses of the ascospore progeny that in all cases the aberrancies were caused by mutation from dependence to independence, and that the original segregation had indeed been 2:2. The surprising feature is the fact that this adenine-requiring mutant had always been quite stable in vegetative (haploid) propagation. It is apparent that there is at least a formal similarity between these results and some of Lindegren's. Some further clue may be forthcoming from the claim by Winge (47) that sucrose and maltose fermentations are controlled by more than one gene. Another possible source of error has been pointed out by Winge and Roberts in a recent article (51). They note that in some cultures a number of asci are observed that have more than the usual number (4) of ascospores. These, they feel have probably arisen as a result of an extra mitotic division. They suggest that in some cases, extra mitoses may occur, but only four nuclei may survive to form ascospores. Under these conditions, it would be a random matter which nuclei survived, and the resulting ascus might, upon analysis, yield highly irregular results. While these may be contributing factors in some of the aberrant ratios which Lindegren has obtained, they cannot easily explain all of them. The problem of aberrant ratios remains one of the most challenging in yeast genetics, and if the findings of Lindegren

and Mundkur are substantiated, they will constitute an important contribution to biology.

Biochemical Mutants

The ability of various agents such as X rays, potassium cyanide, and lithium chloride, to produce variation in yeasts has been reviewed by Henrici (3). More recently, Skovsted (52, 53) has reported morphological variations following treatment with camphor. The production of cultures with increased nutritional requirements after ultraviolet irradiation (54) and nitrogen mustard treatment (50) has been reported in the last two years. A brief discussion of the procedures involved in obtaining such mutant cultures might not be amiss. The techniques that will be outlined here are not necessarily the best that might be devised for mutant production and detection, but could serve as a starting point for such an investigation.

It is obviously an advantage to treat haploid, rather than diploid, cells to produce mutations. In the diploid, there are present two sets of alleles and only dominant mutations of homozygous loci will be phenotypically observable. In the haploid, where there is only one representative of each allele, a recessive mutation (the type most frequently encountered thus far) will be discernible. Hence, the investigator, studying a yeast like S. cerevisiae, would do well first to isolate a haploid clone after sporulation, and then to carry out the mutagenic treatment.

In general, it is desirable to reduce the total population number in order to increase the percentage of mutants. Hence, survival curves are usually run as a preliminary measure, to indicate the dosage necessary to kill a given percent (for example, 99%) of the total population. With these data in hand, cells may be exposed to an appropriate dosage, and then plated on agar in a synthetic minimal medium which will support the growth of the nutritionally wild-type organism. Following the layer plate procedure of Lederberg and Tatum (55), the cells are covered with a layer of

sterile agar so that there are no cells on the top surface of the agar. After suitable incubation period, the plates are marked or photographed (56) so that the colonies which have grown up on the minimal agar are identified. Then another layer of agar is introduced, carrying a nutritional supplement that will permit the growth of deficient cells. The plates are reincubated and the new colonies that appear are isolated for further study.

The colony isolates are now tested for their nutritional status by inoculation into two media: one containing and one lacking the supplement that was used in the original plate layering. If there is growth in the former and none in the latter, the culture may be presumed to be a mutant, differing in its nutritional requirements from the wild-type parent. The determination of the specific nutrilite that the mutant requires is obviously prescribed by the nature of the original supplementation. It might have contained vitamins, amino acids, purines, pyrimidines, etc. depending on what the investigator wished to study. If we take for our example the case where all of the above groups of compounds were added, it would now be necessary to find in which group the nutritional requirement lies, and then to uncover the specific requirement. For a discussion of other types of mutants, the excellent review by Lederberg (57) should be consulted.

Once suitable genetic markers have been introduced into a clone, it may be used in genetic analysis. Pomper and Burkholder (23) have reported results on crosses involving a number of such induced biochemical markers. They found very regular inheritance to be the rule for genes involved in the syntheses of thiamine, tryptophan, uracil, methionine, and adenine. Using the diploid recovery procedure already described (in the section on mating types), these authors were able to differentiate among genetically dissimilar types which were phenotypically similar. To cite the case which they studied most completely, two clones which required uracil for growth were mated. After several days, the mixture of haploids and

diploids were plated on agar lacking uracil, but containing everything else that the haploids required. If the two haploids were allelic with regard to uracil, that is, if in both cases the same gene had been mutated, one would not expect growth in the absence of uracil. Conversely, if growth does occur, it could be taken as presumptive evidence that the same gene was not mutated in both haploids. Growth did occur, and a number of colonies were isolated. When one of these diploid clones was induced to sporulate and the segregants recovered, it was found that the ratios obtained corresponded to those predicted for a two-gene segregation.

Cytology

Yeast cytology is in rather an unsettled state, and so a review of this subject might very profitably be deferred until some clarification is available. There is very considerable debate as to the number of chromosomes or indeed the very identity of the nucleus itself (36, 58). Resolution of this problem may perhaps be forthcoming as genetic data continue to accumulate. We may look forward to chromosome maps of yeast, completely analogous to those of Neurospora, Drosophila, or other forms upon which genetic analyses have been carried out. Lindegren (26) has made a start in this direction, and while his maps are still preliminary, they emphasize the value of further work along these lines.

Conclusions

It seems natural to conclude an article of this sort with a brief look at possible future developments in the field of yeast genetics. The practical breeding possibilities inherent in such work are quite obvious. Although it is true that most of the mutants that have been induced thus far are less vigorous than the parental types, it is quite feasible that with new techniques, new types of mutants may be obtained with more desirable characteristics. The Lindegrens have shown

that it is frequently possible to cross species, and thus incorporate new and valuable qualities into a hybrid. Winge and Laustsen (17) were able to produce a hybrid which was used in the baking industry because of its desirable characteristics of yield and carbon dioxide production. Thus we may add the technique of recombination to that of selection, which has thus far been the main weapon in the yeast industry's armamentariam.

The more controversial portions of yeast genetics will surely continue under vigorous investigation. It is to be hoped that critical experiments will be devised to evaluate the new concepts of gene interaction proposed by the Lindegrens. Since it is possible to do tetrad analysis with ascomycetous fungi like the yeasts, the most unequivocal sort of genetic analyses are possible. For this reason, the theoretical implications of findings from studies of yeast genetics are great. It is certainly true that in some instances it has been possible to demonstrate great regularity of inheritance. In others, it is equally true that very considerable irregularities have been encountered. The question that needs to be resolved is whether our concepts of gene action and interaction must be modified to fit the experimental data, or whether the experimental data actually fit current theory but are confused by experimental artifacts.

Although the biochemical implications of the work with biochemically deficient mutants have not been emphasized in this review, they should not be overlooked. As is now well accepted, the investigations in biochemical genetics with the fungus Neurospora have constituted one of the great biochemical advances of the past decade. Similar possibilities are available to the biochemist working with yeast mutants, and work has already begun along these lines. In many ways, yeast is even better adapted for such work than is Neurospora, since there is a great deal already known about the biochemistry of yeast. We may look forward to a furtherance of this knowledge by studies in biochemical genetics with yeast.

In conclusion, we might refer to a recent statement by Winge (46) who said, with justifiable pride, " ... while a few years ago it was regarded as nonsense to talk about genetics of yeast and hybridization of yeast, it has turned out to be quite as natural as when you speak of genetics and hybridization of flowering plants." Thus, yeast which has long been a favored source of experimental information for the physiologist and the biochemist, is now coming under the scrutiny of the geneticist and we may hope will be as rewarding to him.

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