THE USE OF LINEAR ASCI FOR CHROMOSOME MAPPING IN SACCHAROMYCES

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IN yeast, as in other organisms where tetrad analysis is feasible, it is possible to determine the distance between a given gene and the centromere of its chromosome. This may be accomplished directly if the relationship of the spores in the ascus is known. Two such relationships have been demonstrated. In the four-spored asci of *Schizosaccharomyces pombe*, the two spores at each end of the ascus are sisters (LEUPOLD 1950). An ascogenous cell of genotype *A a* produces an ascus in which spores of composition *A*, *A*, *a*, and *a* are arranged in that order if there has been no recombination between the locus of *A* and the centromere. If a crossover has occurred in this region, the following sequences, indicative of second-division segregation, are obtained: (*A a a A*), (*a A A a*), and (*A a A a*). In *Saccharomycodes ludwigii*, the distribution of the nuclei is such that the two spores at each end of the ascus are nonsisters (WINGE and LAUSTSEN 1939). If the nuclear distribution is random otherwise, the following noncrossover spore arrays would be expected: (*A a a A*), (*a A A a*), and (*A a A a*). If recombination has occurred between *A* and the centromere, the same three arrays would be expected half the time, and a fourth array (*A A a a*) the other half. An estimate of the frequency of second-division segregation for a gene in this organism would therefore be twice the frequency of (*A A a a*) asci. The consequence of a non-random nuclear distribution in *S. ludwigii* will be discussed later.

In linkage studies in Saccharomyces hybrids, it has been customary to dissect asci of the oval type, in which spore relationship is not known. Segregation data obtained from these asci have been used in chromosome mapping by LINDEGREN (1949a, 1951). The equations for calculating gene-centromere linkage in unordered tetrads have been given by PERKINS (1949) and WHITEHOUSE (1950). This method is, however, indirect; in order to determine whether or not a given gene exhibits linkage with the centromere, it is necessary to have segregating in the same asci another gene which exhibits centromere linkage and for which the recombination frequency is known. Saccharomyces hybrids also produce asci which are elongated and in which the spores are arranged in a linear series. Hybrids may be selected which yield as many as 40 percent of linear asci. The investigation reported below was undertaken to determine the pattern of spore array in these asci, for the purpose of ascertaining their usefulness in gene-centromere linkage studies.

TYPES OF SEGREGATIONS EXPECTED IN LINEAR ASCI

When this study of the linear asci of Saccharomyces hybrids was begun, the consequences of three types of nuclear distribution in the ascus after meiosis were considered. They were: (1) the distribution by spindles in a line as in *Schizosaccharomyces*

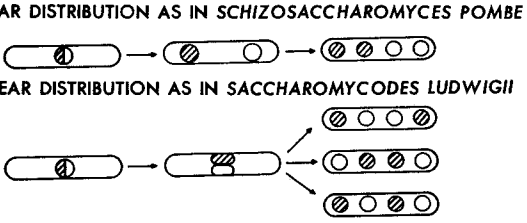

FIGURE 1.—The possible orientations of nuclei in linear asci. The types of asci given for *S. ludwigii* are those expected if the nuclei at each end are non-sisters and are otherwise arranged at random.

pombe, (2) the distribution by parallel spindles as in *Saccharomycodes ludwigii*, and (3) a random distribution resulting from a slippage of the nuclei in the elongated ascogenous cell. The first two distributions, which result in a definite orientation of the nuclei in the linear ascus, are diagrammed in figure 1. A distinction between the three types of nuclear distribution during meiosis is possible by the use of the linear array. As MATHER (1935) has shown, the maximum percentage of second-division segregation, if there is no interference, is 66.7 percent, which is the limit attained with an infinite number of exchanges between the locus and the centromere. This means that if the nuclear distribution is of the *S. pombe* type, for a given gene the frequency of asci with the noncrossover spore array, (*A A a a*), will have a lower limit of 33.3 percent. If the nuclear distribution is by means of parallel spindles, the frequency of asci with the crossover array (*A A a a*) will have an upper limit of 33.3 percent. If there is a random distribution of the nuclei, the spore array (*A A a a*) will be expected for every gene, linked or unlinked with the centromere, in a third of the asci. For a gene showing no centromere linkage, the four spore arrays (*A A a a*), (*A a a A*), (*a A A a*), and (*A a A a*) will be expected in a ratio of 2:1:1:2, regardless of the type of nuclear distribution.

MATERIALS AND METHODS

Preliminary crosses were made to incorporate as many characters as were available into a single hybrid. The hybrid obtained was heterozygous for the genes controlling seven characters that were readily scored and which gave 2:2 segregations in nearly every ascus. These characters were mating type, galactose and melibiose fermentation, and histidine, tryptophane, uracil, and methionine independence.

The yeast stocks were maintained on a nutrient agar containing 1% peptone, 1% yeast extract, and 2% dextrose. For the mating type tests a broth medium of the same composition was used; the segregants were classified as *a* or α as a result of crosses with strains of known mating type. The galactose fermentation tests were made in Durham tubes using a broth of 1% peptone, 1% yeast extract, and 2% galactose. The tests for the fermentation of melibiose were made in Kahn tubes containing 1½ cc of the following medium adjusted to pH 6.0: 1% peptone, 1% yeast extract, 2% melibiose, and the indicator bromcresol-purple. The nutritional requirements were scored in media with a basal composition of Bacto-Vitamin Free Yeast Base, less histidine, tryptophane, and methionine, and with the addition of biotin, pyridoxine hydrochloride, and thiamine hydrochloride, a formula of WICKERHAM's (1953).

Three clones of the hybrid were dissected. Two of these, No. 1002 and No. 1004, were obtained by isolating with the micromanipulator single diploid cells from a mass mating of the haploid parents. The third diploid, No. 1003, was obtained by following the procedure suggested by POMPER AND BURKHOLDER (1949), i.e. by plating the mating mixture on a minimal synthetic medium which lacked the above four essential nutrients and then selecting one of the prototrophic clones.

Linear asci in which the ascus envelopes were slightly longer than that needed to confine the four spores were selected for dissection; a group of suitable asci is pictured in plate I-F. The order of the spores in the ascus was retained by cutting the tip of the ascus and then squeezing out the spores one at a time for transfer to droplets of nutrient medium. If, during a dissection, a spore was crushed or the order of the spores was lost, the ascus was abandoned.

RESULTS

From about 150 dissections of the hybrid resulting from the cross of haploid parents of these genotypes, (*a g ME HI tr MT ur*) and (*α G me hi TR mt UR*), 74 asci were obtained from which all four spores germinated and produced colonies that could be transferred and tested. In the 74 asci, the characters mating type, galactose and melibiose fermentation, histidine, tryptophane, and methionine independence gave the expected 2:2 ratios in every instance. For uracil independence, there were three asci with irregular ratios and these were not included in the analysis of the segregations for this character.

In order to detect linkage and to apply the equations of WHITEHOUSE (1950) to determine centromere linkage in unordered tetrads, the characters were considered in pairs. When the segregations for the genes two at a time are studied, there are three categories for the asci based on the genotypes of the spores. Following the terminology of PERKINS (1953), a diploid hybrid of genotype (*A B /a b*) will yield parental ditype asci (*AB AB ab ab*), non-parental ditype asci (*Ab Ab aB aB*), and tetratype asci (*AB Ab aB ab*). In table 1 the possible pairings of the seven characters are scored as to the three types of asci and in addition the proportion of the tetratype asci is calculated. The data in table 1 indicate that there is no close linkage between any of the genes since there is no preponderance of the parental ditype asci for any of the combinations. From the consideration of the proportion of tetratype asci, it is also apparent that no two of the genes are closely linked to the centromere, since centromere linkage for a pair of genes would be indicated by a proportion of tetratype asci significantly less than two thirds. In every instance, the fraction is so nearly two thirds that it is impracticable to use the equations for the determination of centromere linkage in unordered tetrads.

When the criterion of spore array was applied to the same data, evidence of close linkage with the centromere was obtained for the tryptophane locus. The asci were scored as to spore array, (*A A a a*), (*A a a A*), (*a A A a*), or (*A a A a*), for each character. In the segregations for tryptophane independence, 70 of 74 asci were of the fourth array (table 2). This was interpreted as evidence that the fourth array, (*A a A a*), is the noncrossover class and that the gene *TR* is closely linked to the centromere of its chromosome. This would mean that the distribution of the nuclei during meiosis is as diagrammed in the last series for *S. ludwigii* in figure 1, with

TABLE 1

Tetrad analysis of the diploid hybrids, 1002, 1003, and 1004 to determine gene-to-gene and gene-to-centromere linkage

Gene pair	Ditype asci		Tetratype asci	Proportion tetratype
	Parental	Non-parent		
Tryptophane-Mating type	22	10	42	.57
Tryptophane-Galactose	15	11	48	.65
Tryptophane-Melibiose	9	15	50	.68
Tryptophane-Histidine	9	8	57	.77
Tryptophane-Uracil	13	13	45	.63
Tryptophane-Methionine	12	11	51	.69
Mating type-Galactose	14	12	48	.65
Mating type-Melibiose	11	16	47	.64
Mating type-Histidine	12	15	47	.64
Mating type-Uracil	10	18	43	.61
Mating type-Methionine	8	13	53	.72
Galactose-Melibiose	15	6	53	.72
Galactose-Histidine	12	13	49	.66
Galactose-Uracil	10	6	55	.78
Galactose-Methionine	11	10	53	.72
Melibiose-Histidine	14	8	52	.70
Melibiose-Uracil	14	12	45	.63
Melibiose-Methionine	19	13	42	.57
Histidine-Uracil	12	7	52	.73
Histidine-Methionine	8	20	46	.62
Uracil-Methionine	8	19	44	.62

TABLE 2

The segregations of the diploid hybrids, 1002, 1003, and 1004 classified according to type of array found in the ascus

Locus	Spore arrays				Percent array IV (noncrossovers)
	I + + − −	II + − − +	III − + + −	IV + − + −	
Tryptophane	1	1	2	70	95
Mating type	26	6	11	31	42
Galactose	20	16	14	24	32
Melibiose	25	18	9	22	30
Histidine	19	16	23	16	22
Uracil	24	11	11	25	35
Methionine	29	13	10	22	30

non-sister nuclei alternating in the ascogenous cell. A single crossover would give an ascus with one of these three arrays, (*A A a a*), (*A a a A*), or (*a A A a*), which are the second division segregation classes. Since the noncrossover class is limited to a single spore array, (*A a A a*), these asci can be conveniently used to determine centromere linkage. For the loci other than the tryptophane locus, there was no evidence of centromere linkage.

On the above assumptions, the gene *TR* can be placed 2.5 units from the centromere since about five percent of the asci indicated second-division segregation. Confirmatory evidence of this close centromere linkage was obtained in tetraploid material, in which linkage with the centromere can be determined for individual loci (ROMAN, PHILLIPS, and SANDS 1955).

The use of tetraploid hybrids to determine centromere linkage for the gene controlling tryptophane independence

A tetraploid hybrid of the genotype, *A A a a*, will produce asci which show 4:0, 3:1, and 2:2 segregations for the phenotype of the dominant allele. The frequency of these segregations may be predicted for a character when the percentage of second-division segregation is known. For a gene which shows five percent second-division segregation in the diploid hybrid, the following percentages for the three types of asci from the tetraploid will be expected if there is bivalent pairing of the homologous chromosomes during meiosis: 4:0 asci 63.3 percent, 3:1 asci 6.7 percent, and 2:2 asci 30 percent. If there is tetravalent pairing, the following percentages will be expected: 4:0 asci 63.5 percent, 3:1 asci 5.4 percent, and 2:2 asci 31.1 percent.

For the 48 asci of the tetraploid hybrid No. 1076, which was of the genotype *TR TR tr tr*, the following segregations were obtained: 28 (4:0) asci, 2 (3:1) asci, and 18 (2:2) asci. The expected numbers on the basis of bivalent pairing and a second-division segregation frequency of five percent are 30.2 (4:0) asci, 3.4 (3:1) asci, and 14.4 (2:2) asci. If pairing is tetravalent, the expected numbers are nearly the same, i.e., 30.5 (4:0) asci, 2.6 (3:1) asci, and 14.9 (2:2) asci. A chi-square analysis shows that the observed frequencies of the segregations for tryptophane independence are in good agreement with the frequencies expected if *TR* is 2.5 units from the centromere, as the evidence based on the linear array indicates.

Cytological evidence on the distribution of the nuclei during meiosis in Saccharomyces hybrids

A tetraploid clone, No. 1F5, was chosen for a cytological study; since upon sporulation, 30 to 40 percent of the asci were linear and the cells were about half again as large as the average diploid cell. To induce sporulation the procedure suggested by LINDEGREN (1949b) was followed. Cells from a two days growth on presporulation medium were transferred to a gypsum slant. After 20 to 24 hours on the slant, the ascogenous cells were placed on cover slips and fixed in osmic acid vapors and Schaudinn's fluid, following the procedure ROBINOW (1944) recommended for nuclear stains of bacteria. The fixed cells were then treated with ribonuclease for about an hour and a quarter at 55°C, and then stained for a half hour with Giemsa stain. Some of the cells that were photographed were mounted in phosphate buffer. It was found however, that in many cases a quick passage through alcohol solutions and mounting in "Euparal" greatly intensified the staining of the nuclei.

No meiotic figures could be discerned although the nuclei were comparatively large as can be seen in plate I-A, which shows a typical elongated ascogenous cell with a single nucleus. When two nuclei were seen, they were always found together near the center of the elongated ascogenous cell in the position indicated in plate I-B. In many

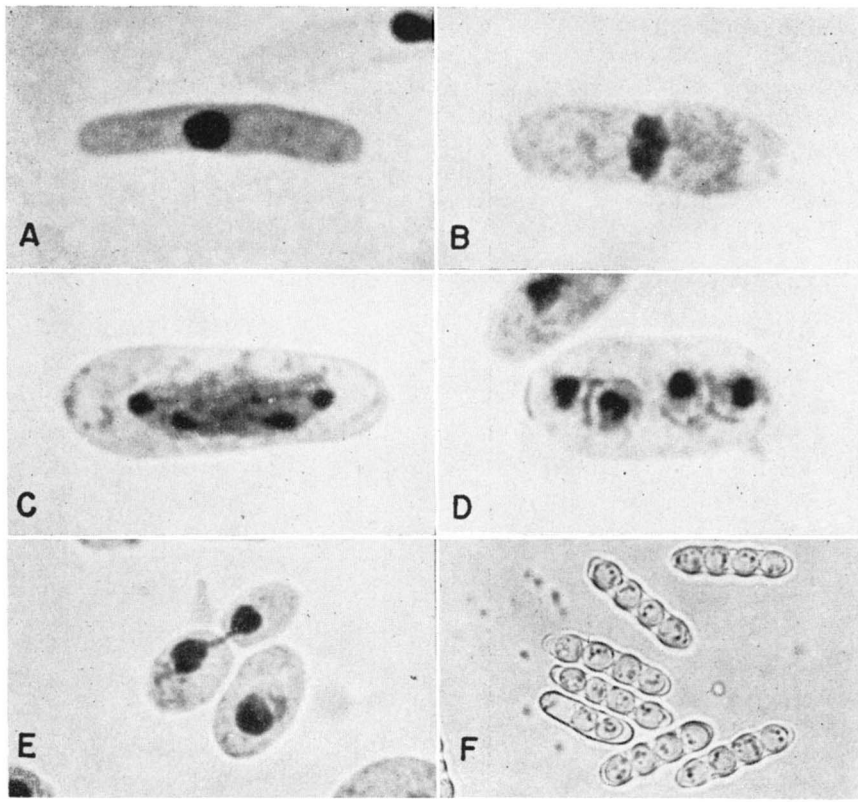

PLATE I.—A-D, ascogenous cells of a Saccharomyces hybrid, Giemsa stain. E, vegetative cells, Giemsa stain. F, linear asci, unstained.

cells in which four nuclei were seen, a thin strand of chromatinic material connecting the sister nuclei was observed, so there was no doubt as to the relationship of the nuclei. An example of this is seen in plate I-C, which shows the alternation of the non-sister nuclei in the ascogenous cell. The final alignment of the four nuclei in the elongated ascogenous cell, just prior to the formation of the spores, is seen in plate I-D. In a thorough inspection of many preparations of this hybrid, No. 1F5, and of other tetraploid and diploid Saccharomyces hybrids from our stocks, stained with Giemsa and by the Feulgen technique, there never were seen any nuclear configurations that would indicate a nuclear distribution like that found in *Schizosaccharomyces pombe*.

The elongated ascogenous cells were found only in material which had been induced to sporulate. The vegetative cells were typically oval and quite regular. In preparations of vegetative cells that were made to illustrate this, no elongated or multinucleated cells were found. A typical mother cell with a bud and a strand of chromatinic material connecting the nuclei of the two is seen in plate I-E.

DISCUSSION

The results from the scoring of tryptophane independence in 74 linear four-spored asci of a diploid Saccharomyces hybrid indicated that the nuclear distribution during

meiosis resulted in the alternation of non-sister nuclei in the elongated ascogenous cell. This manner of distribution is similar to and may be the same as the nuclear distribution in *Saccharomycodes ludwigii*; for in the description by WINGE AND LAUSTSEN (1939) it was proposed only that the nuclear distribution for this organism was by means of parallel spindles with no stipulation as to the final alignment of the nuclei other than that the two spores at one end of the ascus contained non-sister nuclei.

Of the seven genes that were heterozygous in the diploid hybrid, only one, *TR*, showed close centromere linkage. For one other gene, mating type, 58 percent of the asci were of the second-division segregation arrays. The deviation of the observed number of second-division segregation asci, 43, from the number expected for a gene showing no centromere linkage, 49, is not significant at a 95% level. However, when the data for mating type from all crosses where linear asci were dissected are summarized, the following figures are obtained: Array I, 141 asci; Array II, 68 asci; Array III, 88 asci; and Array IV, 261 asci. For the 558 asci, the observed number, 297 asci, showing second division segregation differs significantly from the 372 asci expected if this locus showed no centromere linkage. In this compilation, 53.3 percent of the asci show second-division segregation. LINDEGREN (1949a), using unordered tetrads, has mapped the locus of the mating type gene at 24 units from the centromere. If with the above data, the centromere linkage for the locus of mating type is taken as one half the percentage of second-division segregation, the value obtained, 27 units, is in reasonable agreement with LINDEGREN's figure.

It had been anticipated that the gene for galactose fermentation would show close centromere linkage since LINDEGREN (1949a) has this locus mapped at 6 units from the centromere. Since the galactose gene of this hybrid showed no linkage with the centromere, this was a point of complete disagreement until crosses made with a galactose-negative haploid, supplied through the courtesy of DR. LINDEGREN, showed that we were dealing with two different galactose genes. We have designated our locus as galactose-2. A summary of the data from 348 linear asci indicates 70.7 percent second-division segregation for galactose-2.

A possible source of error in the use of the spore array in centromere mapping would occur if there were an occasional failure of the non-sister nuclei to alternate in the ascus. Such an ascus would be improperly scored as to second-division segregations. The chance for an error of this type, however, is negligible, in this series of dissections at least, since, as indicated by the scoring for tryptophane independence, there would be at most only four possible deviations from the normal nuclear distribution in these 74 asci.

The extra care required to dissect out the spores in order from a linear ascus is justified since the spore arrays provide a direct measure of the degree of second-division segregation while only an estimate of this may be obtained by using the equations for determining centromere linkage in unordered tetrads.

SUMMARY

On the basis of genetic evidence, it was concluded that the nuclear distribution in the linear four-spored asci of a diploid Saccharomyces hybrid follows a definite pattern with the alternation of spores with non-sister nuclei in the ascus. A cytological

investigation of the nuclear distribution during meiosis also indicated that there was an alternation of non-sister nuclei in the elongated ascogenous cell. Thus, a deviation from the alternation of the dominant and recessive characters in the scoring of these asci indicates the occurrence of a crossover and gives a simple and direct means to determine the linkage of a gene to the centromere of its chromosome.

From the analysis of 74 linear asci of the diploid hybrid, the gene for tryptophane independence was mapped at about 2.5 units from the centromere, a value which was confirmed independently in tetraploid material. Evidence is also given that the mating type locus is approximately 27 units from the centromere. The genes for melibiose and galactose (*G*-2) fermentation, histidine, uracil, and methionine independence did not show centromere linkage.

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LITERATURE CITED

LEUPOLD, U., 1950 Die Vererbung von Homothallie und Heterothallie bei *Schizosaccharomyces Pombe*. Compt. Rend. Trav. Lab. Carlsberg Ser. Physiol. **24:** 381–480.

LINDEGREN, C. C., 1949a Chromosome maps of Saccharomyces. Hereditas Suppl. Vol. (Proc. Inter. Congr. Genet.) 338–355.

1949b *The Yeast Cell, Its Genetics and Cytology*. St. Louis, Educational Publishers Inc.

LINDEGREN, C. C., and GERTRUDE LINDEGREN, 1951 Linkage relations in Saccharomyces of genes controlling the fermentation of carbohydrates and the synthesis of vitamins, amino acids and nucleic acid components. Ind. Phyto. **4:** 11–20.

MATHER, K., 1935 Reductional and equational separation of the chromosomes in bivalents and multivalents. J. Genet. **30:** 53–78.

PERKINS, D. D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:** 607–626.

1953 The detection of linkage in tetrad analysis. Genetics **38:** 187–197.

POMPER, S., and P. R. BURKHOLDER, 1949 Studies on the biochemical genetics of yeast. Proc. Nat. Acad. Sci. U.S. **35:** 456–464.

ROBINOW, C., 1944 Cytological observations on *Bact. Coli, Proteus vulgaris* and various aerobic spore-forming bacteria with special reference to nuclear structures. J. Hyg. **43:** 413–423.

ROMAN, H., M. M. PHILLIPS, and S. M. SANDS, 1955 Studies of polyploid Saccharomyces. I. Tetraploid segregation. Genetics **40:** 546–561.

WHITEHOUSE, H. L. K., 1950 Mapping chromosome centromeres by the analysis of unordered tetrads. Nature **165:** 893.

WICKERHAM, L. J., 1953 Formula for the vitamin free yeast base, p. 251 in the Difco Manual, ninth edition. Detroit, Difco Laboratories.

WINGE, O., and O. LAUSTSEN, 1939 *Saccharomycodes Ludwigii* Hansen, a balanced heterozygote. Compt. Rend. Trav. Lab. Carlsberg Ser. Physiol. **22:** 357–370.