

# GENETIC MAPPING IN SACCHAROMYCES

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**I**N our first report of linkage relationships of centromere-linked genes in *Saccharomyces*, 25 genes were located on ten chromosomes (HAWTHORNE and MORTIMER 1960). Three additional chromosomes have since been proposed by LINDEGREN, LINDEGREN, SHULT, and HWANG (1962); and HWANG, LINDEGREN, and LINDEGREN (1963, 1964). The present work extends the linkage maps of this yeast by providing evidence for 14 linkage groups as well as additional linkages on the established chromosomes. Linkage groups that may represent still other chromosomes also are described.

## MATERIALS AND METHODS

The genes included in this report are listed in Table 1. A description of these loci is given in the report of the Carbondale Yeast Genetics Conference (1963). The techniques for the isolation and sporulation of diploid hybrids, and the isolation and analysis of spore tetrads, have been described (HAWTHORNE and MORTIMER 1960). Only complete tetrads are included in the analyses.

To the tests and media described in the previous paper can be added actidione resistance scored at a concentration of 1.3  $\mu\text{g}/\text{ml}$  in synthetic medium.

Since the hybrids used were frequently heterozygous for 20 or more genes, replica plating techniques were employed to reduce the labor required to score the characters. In most hybrids there were several cases where two or more genes controlled a given phenotype. For these combinations it was necessary to perform intergenic complementation tests on the spore progeny. Secondary master plates, cross-stamped with appropriate tester strains of both mating types, were incubated for one day to permit mating and then replica-plated onto the diagnostic media to score the segregation of the individual complementing genes.

*Marker localization:* In addition to standard tetrad analysis procedure, discussed below, two additional techniques were of considerable value in locating genes on the linkage map. One of the techniques involved the use of disomic test stocks. If the hybrid of the mutant and disomic stocks is of constitution  $A/A/a$ , then asci with aberrant segregation ratios, 4:0 and 3:1, will predominate. The observation of only 2:2 ratios establishes that the marker is not on the chromosome that is disomic. In practice, a normal haploid carrying a number of unmapped genes is crossed to the disomic to increase the efficiency of this method. Because the disomic cultures are relatively unstable and tend to lose the extra chromosome, it is necessary to construct the hybrid with one genetic marker known to be on the chromosome in duplex condition to confirm the presence of the extra chromosome.

Markers can be restricted to a given chromosome arm and their sequence determined by mitotic segregation studies. Such techniques have been used extensively with *Aspergillus* (PONTECORVO and KÄPER 1958). In yeast, sectoring of colonies formed by irradiated heterozygous diploid cells was first described by JAMES and LEE-WHITING (1955). ROMAN (1956) has analyzed mitotic segregants that occur spontaneously in diploid yeast heterozygous for various adenine

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color markers. In the majority of cases, it has been established that segregation occurs through mitotic crossing over. Concomitant sectoring of markers is a criterion of linkage, and the frequency with which concomitance occurs is a measure of the degree of linkage and indicates the position of a given marker, proximal or distal, with reference to another.

Indications of linkage with the above procedures are pursued by conventional tetrad analysis. In tetrad analysis, the statistic employed to determine if two genes *A* and *B* are linked in the cross  $AB \times ab$  is the ratio of parental ditype (PD) asci,  $AB AB ab ab$ , to nonparental ditype (NPD) asci,  $Ab Ab aB aB$ , to tetratype (T) asci,  $AB Ab aB ab$ . Linkage is indicated when the PD:NPD ratio is significantly greater than one. The distance  $x$ , in centimorgans, between the two genes can be obtained from the equation,  $x = [(T + 6NPD) \times 100] / [2(PD + NPD + T)]$  (PERKINS 1949).

Although unordered tetrads generally are isolated for genetic analysis in *Saccharomyces*, it is possible to determine the first-division segregation pattern in individual asci when several known centromere markers are included in the cross (HOWE 1956; HAWTHORNE and MORTIMER 1960). A new marker is accepted as centromere-linked if its second-division segregation (SDS) frequency is significantly less than two-thirds. An SDS frequency in excess of  $\frac{2}{3}$  is considered to be the consequence of strong chiasma interference. Markers found to be centromere-linked were tested for linkage against known centromere markers.

## RESULTS

*Centromere linkage:* A compilation of the second-division segregation frequencies of 102 genes is given in Table 1. One third (34/102) of these genes exhibit centromere linkage. Sixty percent second-division segregation was taken as a convenient value for the demarcation of the centromere-linked class. A gene could be misclassified on the basis of this criterion either because of statistical fluctuations or because of chiasma and chromatid interference patterns that would lead to SDS frequencies below  $\frac{2}{3}$  for genes considerably removed from the centromere.

Our first map included the following centromere markers: I-*ad*<sub>1</sub>, II-*ga*<sub>1</sub>, III-*α* and *hi*<sub>1</sub>, IV-*tr*<sub>1</sub> and *ga*<sub>3</sub>, V-*ur*<sub>3</sub>, VI-*hi*<sub>2</sub>, VII-*le*<sub>1</sub>, *tr*<sub>5</sub>, and *ad*<sub>6</sub>, VIII-*p*<sub>1</sub>, *ar*<sub>4</sub>, and *thr*<sub>1</sub>, IX-*hi*<sub>6</sub> and *ly*<sub>1</sub>, and X-*met*<sub>2</sub>. However, we concur in the assessment by LINDEGREN *et al.* (1962) that the use of *met*<sub>2</sub> as a centromere marker defining a new linkage group was premature. In its stead, they proposed that another methionine gene, *met*<sub>3</sub>, be adopted as the centromere marker of chromosome X (LINDEGREN *et al.* 1962). HWANG *et al.* (1963) showed that *met*<sub>14</sub> segregated independently from the above centromere markers and thus represented a new chromosome, XI. A twelfth chromosome was also proposed by HWANG *et al.* (1964) as containing the markers *thr*<sub>5</sub> and *ly*<sub>7</sub>. Our own studies of hybrids heterozygous for *thr*<sub>5</sub> and *ly*<sub>7</sub> show that these two genes not only assort independently with respect to the centromere markers of linkage groups I through XI but are themselves unlinked (Tables 3, 4). Accordingly, we have retained *thr*<sub>5</sub> for linkage group XII and have assigned *ly*<sub>7</sub> to the centromere of linkage group XIII. Another new linkage group, XIV, is established with the demonstration that *p*<sub>8</sub> is unlinked to all the above centromere markers (Tables 3, 4). The remaining centromere-linked genes from Table 1 have been mapped on the above linkage groups: II, *ac*<sub>1</sub><sup>c</sup> (DESBOROUGH, SHULT, YOSHIDA, and LINDEGREN 1960), *p*<sub>9</sub>, *ga*<sub>7</sub>, and *ga*<sub>10</sub> (DOUGLAS and HAWTHORNE 1964); III, *le*<sub>2</sub> (LINDEGREN *et al.* 1962);

TABLE 1

*Second-division segregation frequencies of 102 genes in Saccharomyces*

Gene	Division segregation			Gene	Division segregation			Gene	Division segregation		
	First	Second	% second		First	Second	% second		First	Second	% second
$\alpha$	1463	1015	41.0	$is_3$	98	13	10.8	$py_2$	44	137	75.1
$ac_1^r$	123	30	19.6	$le_1$	1518	78	4.9	$pn_1$	40	91	69.5
$ac_2^r$	29	81	73.5	$le_2$	244	36	12.9	$ROC_1$	72	76	51.4
$ac_3^r$	52	13	20.0	$ly_1$	141	179	55.9	$ser_1$	71	144	67.0
$ac_4^r$	94	173	64.8	$ly_2$	180	381	67.9	$S_a$	36	66	64.7
$ad_1$	1177	131	10.0	$ly_5$	69	108	61.8	$S_d$	260	10	3.7
$ad_2$	210	661	75.9	$ly_6$	16	28	63.6	$S_h$	35	72	67.3
$ad_3$	54	130	70.6	$ly_7$	384	340	49.4	$S-ser_1$	39	43	52.5
$ad_4$	47	94	66.7	$ly_9$	33	28	46.8	$s-met_1$	37	70	65.4
$ad_{5,7}$	83	171	67.4	$MA_1$	90	212	70.2	$SU_1$	33	64	66.0
$ad_6$	355	371	51.1	$MA_2$	92	197	68.2	$SU_2$	204	369	64.4
$ad_8$	154	291	65.4	$MA_3$	211	563	72.7	$SU_3$	66	100	60.2
$ar_1$	20	34	63.0	$MA_4$	18	65	78.5	$SU_4$	67	146	68.5
$ar_4$	935	189	16.8	$MA_5$	28	77	73.3	$SU_5$	19	39	67.2
$ar_{5,6}$	27	44	60.5	$MEL$	305	788	72.1	$SU_6$	28	53	65.4
$ar_8$	74	155	67.7	$met_1$	197	497	71.6	$th_1$	56	84	60.0
$ar_9$	72	5	6.5	$met_2$	269	446	62.3	$thr_1$	288	234	44.8
$cana_1$	27	86	76.1	$met_3$	174	13	7.5	$thr_2$	78	172	68.8
$CU_1$	155	488	75.9	$met_4$	57	132	69.8	$thr_3$	83	164	66.4
$ga_1$	981	150	13.3	$met_5$	20	54	73.0	$thr_4$	100	202	66.9
$ga_2$	145	424	74.5	$met_6$	64	136	68.0	$thr_5$	129	48	27.1
$ga_3$	95	2	2.1	$met_{10}$	44	68	60.7	$thr_6$	26	49	65.3
$ga_4$	109	180	62.2	$met_{14}$	133	4	2.9	$tr_1$	2112	20	0.94
$ga_5$	84	155	64.9	$MG_1$	42	63	60.0	$tr_2$	95	235	71.2
$ga_7$	548	72	11.6	$MG_2$	71	131	64.8	$tr_3$	16	35	68.6
$ga_{10}$	159	20	12.6	$MG_3$	48	90	65.2	$tr_4$	58	99	63.1
$hi_1$	104	200	65.8	$p_1$	559	49	8.1	$tr_5$	389	188	32.6
$hi_2$	702	404	36.5	$p_2$	46	108	70.1	$ty_1$	123	224	64.6
$hi_4$	305	217	41.6	$p_3$	86	191	69.0	$ty_{2,4}$	123	265	68.3
$hi_5$	63	157	71.4	$p_4$	22	36	62.1	$ty_3$	74	123	62.5
$hi_6$	418	191	31.4	$p_6$	34	61	64.2	$ur_1$	263	516	66.2
$hi_7$	60	144	70.6	$p_8$	242	0	0	$ur_2$	76	203	72.8
$hi_8$	269	484	64.2	$p_9$	108	62	36.5	$ur_3$	512	58	10.2
$is_1$	12	35	74.5	$pha_2$	113	182	61.7	$ur_4$	87	217	71.5

The symbols refer to the following phenotypes: *a*/ $\alpha$  mating-type gene; nutritional requirements: *ad*- adenine, *ar*- arginine, *hi*- histidine, *is*- isoleucine or isoleucine plus valine, *le*- leucine, *ly*- lysine, *met*- methionine, *ph*- phenylalanine, *py*- pyridoxine, *pn*- pantothenate, *ser*- serine, *th*- thiamine, *thr*- threonine (*thr*<sub>1</sub> and *thr*<sub>4</sub>) or threonine plus methionine (*thr*<sub>2</sub>, *thr*<sub>3</sub>, *thr*<sub>5</sub>, and *thr*<sub>6</sub>), *tr*- tryptophan, *ty*- tyrosine (*ty*<sub>1</sub>), tyrosine plus phenylalanine (*ty*<sub>2,4</sub>) or tyrosine plus phenylalanine plus tryptophan (*ty*<sub>3</sub>), and *ur*- uracil; resistance genes: *ac*- actidione, *cana*- canavine, *CU*- copper; *ROC*- roccal; carbohydrate utilization: *ga*- galactose nonfermenter, *MA*- maltose fermentation, *MEL*- melibiose, *MG*- alpha-methyl glucoside, *SU*- sucrose; genetic petite loci: *p*; suppressors: *S*<sub>a</sub> to *S*<sub>h</sub>- super suppressors, (HAWTHORNE and MORTIMER 1963), *S-ser*<sub>1</sub> and *s-met*<sub>1</sub>- specific suppressors. The subscripts (number or letter) identify particular genetic loci.

V, *S-ser*<sub>1</sub> and *ar*<sub>9</sub> (Table 2); VI, *met*<sub>10</sub> (LINDEGREN *et al.* 1962) and *S*<sub>d</sub> (Table 2); VII, *ac*<sub>3</sub><sup>r</sup> (Table 2); X, *is*<sub>3</sub> (Table 2); XII, *ROC*<sub>1</sub><sup>r</sup> (Table 2); and XIV, *ly*<sub>9</sub> (Table 2).

*Additional linkages:* Trisomic analysis provides unambiguous evidence for linkage of markers that recombine too freely to be identified as linked by tetrad

analysis. The validity of the technique has been demonstrated by ROMAN, PHILLIPS and SANDS (unpublished) who showed that the genes  $\alpha$ ,  $hi_1$ ,  $ga_2$ ,  $SU_2$ , and  $ur_1$  were on different chromosomes. In addition, the linkage of  $hi_1$  and  $tr_2$  (LINDEGREN and LINDEGREN 1951) was demonstrated by this technique, and one other pair of genes,  $ur_1$  and  $me_1$ , were located on another chromosome. Additional stocks, disomic for the chromosomes I, II, VII, or VIII, were included in another attempt to locate new mutants (TAKAHASHI and HAWTHORNE, unpublished). The location of genes already mapped on these chromosomes was confirmed, and  $hi_7$  was found to be on linkage group II. The linkage of  $ur_1$  and  $me_1$  was verified and linkage groups I through V, VII, VIII, IX, and XII were eliminated as possibilities for the location of this linkage group.

The linkages and order of the loci  $c$  (centromere)– $ad_6$ – $MA_1$ – $ad_3$ ,  $c$ – $ac_2^+$ – $ly_5$ – $ad_{5,7}$ ,  $c$ – $ty_2$ – $thr_2$ – $tr_4$ – $ad_8$ , and  $c$ – $ad_2$ – $ser_{11}$ – $hi_8$ , were originally established by mitotic recombination studies (ROMAN 1956; NAKAI and MORTIMER, in preparation; JOHNSTON and MORTIMER, in preparation; GILMORE, unpublished) and then confirmed in part by tetrad analysis (Table 2). The first two groups proved to be on the two arms of linkage group VII. The other two groups are either on unmapped arms of the identified linkage groups or represent new linkage groups.

Miscellaneous cases of linkage have been discovered by tetrad analysis. They have been appended to the list in Table 2 and included in the maps when three or more loci are involved. For two of the longer linkage groups,  $pha_2$ – $met_2$ – $p_2$  (Table 2, Fragment 3) and  $SU_2$ – $hi_8$ – $ly_{11}$  (LINDEGREN *et al.* 1962; and Table 2, Fragment 4), mitotic segregation techniques were used to determine the proximal markers,  $pha_2$  and  $SU_2$ . The segregation of these two genes relative to the centromere markers gave no tetrad ratios indicative of linkage.

*Linkage maps:* Maps based on the data in Tables 1 to 4 are presented in Figure 1. For all linkage groups, the genes have been ordered by the analysis of crosses that segregated for all or most of the genes on the particular linkage group. Generally, the order was apparent from the relative lengths of the gene-gene or gene-centromere intervals and consistent sums or differences were observed. On occasion, however, it was necessary to inspect the crossover patterns in individual asci and choose the sequence which minimized the number of multiple exchanges.

The order is still ambiguous in several clusters of genes. Mitotic recombination experiments have been used in placing  $ga_{10}$  proximal to  $ga_1$ , but the position of  $ga_7$  in this cluster has not been determined. Thus far, crosses involving these three genes have not yielded tetrads with meiotic exchanges. Other cases where no or negligible crossing over has hindered the mapping efforts are: centromere IV– $tr_1$ – $ga_3$ ,  $Su_1$ – $MA_1$ ,  $SU_8$ – $MA_8$ – $MG_2$ , and  $p_{11}$ – $ty_1$ .

#### DISCUSSION

Early in our mapping program it became apparent from the paucity of cases of linkage that either yeast has a large number of chromosomes or that the linkage groups were very long. Subsequent studies have shown that both factors were

TABLE 2

*Tetrad data indicating gene to gene linkage (for description of gene symbols see footnote to Table 1)*

Linkage group	Gene pair	PD	NPD	T	Linkage group	Gene pair	PD	NPD	T	
II	<i>p<sub>9</sub>-ac<sub>1</sub><sup>r</sup></i>	32	0	19	VIII	<i>le<sub>1</sub>-ad<sub>6</sub></i>	208	9	290	
	<i>p<sub>9</sub>-ga<sub>1</sub></i>	72	0	70		<i>ad<sub>6</sub>-MA<sub>1</sub></i>	14	15	49	
	<i>ac<sub>1</sub><sup>r</sup>-ga<sub>1</sub></i>	93	1	52		<i>MA<sub>1</sub>-SU<sub>1</sub></i>	108	0	0	
	<i>ga<sub>1</sub>-ga<sub>7</sub></i>	313	0	0		<i>MA<sub>1</sub>-ad<sub>3</sub></i>	42	7	89	
	<i>ga<sub>1</sub>-ga<sub>10</sub></i>	59	0	0		<i>p<sub>1</sub>-ar<sub>3</sub></i>	371	0	30	
	<i>ga<sub>7</sub>-ga<sub>10</sub></i>	72	0	0		<i>ar<sub>3</sub>-thr<sub>1</sub></i>	453	0	138	
	<i>ga<sub>1</sub>-ly<sub>2</sub></i>	101	23	259		<i>thr<sub>1</sub>-CU<sub>1</sub></i>	259	2	225	
	<i>ly<sub>2</sub>-ty<sub>1</sub></i>	129	6	200		<i>CU<sub>1</sub>-p<sub>3</sub></i>	88	4	148	
	<i>p<sub>11</sub>-ty<sub>1</sub></i>	17	0	0		IX	<i>hi<sub>6</sub>-ly<sub>1</sub></i>	138	17	256
	<i>ty<sub>1</sub>-met<sub>8</sub></i>	87	0	42			X	<i>is<sub>3</sub>-met<sub>3</sub></i>	69	0
	<i>met<sub>8</sub>-hi<sub>7</sub></i>	53	0	35		XII	<i>thr<sub>5</sub>-ROC<sub>1</sub><sup>r</sup></i>	53	0	5
	<i>ty<sub>1</sub>-hi<sub>7</sub></i>	34	3	90			<i>thr<sub>5</sub>-ga<sub>2</sub></i>	32	0	31
III	<i>hi<sub>1</sub>-le<sub>2</sub></i>	353	3	165	XIV	<i>p<sub>8</sub>-ly<sub>9</sub></i>	24	2	25	
	<i>le<sub>2</sub>-α</i>	209	12	260		Frag. 1	<i>hi<sub>8</sub>-ser<sub>1</sub></i>	249	0	68
	<i>α-thr<sub>3</sub></i>	268	4	162	<i>hi<sub>8</sub>-ad<sub>9</sub></i>	16	0	5		
	<i>thr<sub>4</sub>-MA<sub>2</sub></i>	137	4	145	<i>ad<sub>9</sub>-ad<sub>2</sub></i>	23	0	16		
IV	<i>ga<sub>3</sub>-tr<sub>1</sub></i>	123	0	1	<i>ser<sub>1</sub>-ad<sub>2</sub></i>	100	1	109		
	<i>tr<sub>1</sub>-s-met<sub>1</sub></i>	17	1	32	<i>hi<sub>8</sub>-ad<sub>2</sub></i>	90	4	198		
V	<i>S-ser<sub>1</sub>-ur<sub>3</sub></i>	41	2	41	Frag. 2	<i>ad<sub>2</sub>-ac<sub>4</sub><sup>r</sup></i>	107	5	124	
	<i>ur<sub>3</sub>-ar<sub>9</sub></i>	52	0	9		<i>ty<sub>9</sub>-thr<sub>2</sub></i>	329	0	90	
	<i>ar<sub>9</sub>-thr<sub>3</sub></i>	14	1	21		<i>thr<sub>2</sub>-S<sub>f</sub></i>	46	0	4	
	<i>ur<sub>3</sub>-thr<sub>3</sub></i>	73	2	131		<i>ty<sub>2</sub>-S<sub>f</sub></i>	36	0	22	
	<i>thr<sub>3</sub>-hi<sub>1</sub></i>	38	0	3		<i>thr<sub>2</sub>-tr<sub>4</sub></i>	18	11	52	
	<i>hi<sub>1</sub>-ar<sub>6</sub></i>	63	0	9		<i>tr<sub>1</sub>-ad<sub>8</sub></i>	32	0	46	
	<i>hi<sub>1</sub>-is<sub>1</sub></i>	49	0	20	Frag. 3	<i>pha<sub>2</sub>-met<sub>2</sub></i>	60	1	87	
	<i>is<sub>1</sub>-tr<sub>2</sub></i>	56	0	13		<i>met<sub>2</sub>-p<sub>2</sub></i>	102	0	33	
	<i>ar<sub>6</sub>-tr<sub>2</sub></i>	27	0	14	Frag. 4	<i>SU<sub>2</sub>-hi<sub>5</sub></i>	11	1	19	
	<i>S<sub>d</sub>-hi<sub>2</sub></i>	65	1	41		<i>hi<sub>5</sub>-ly<sub>11</sub></i>	24	0	14	
	VI	<i>hi<sub>2</sub>-met<sub>10</sub></i>	77	0	49	Frag. 5	<i>MA<sub>4</sub>-met<sub>1</sub></i>	40	3	37
		<i>ad<sub>5,7</sub>-ly<sub>5</sub></i>	28	9	59		<i>met<sub>1</sub>-ur<sub>1</sub></i>	53	55	201
VII	<i>ad<sub>5,7</sub>-ty<sub>3</sub></i>	39	17	110	Frag. 6	<i>ur<sub>1</sub>-tr<sub>3</sub></i>	42	0	5	
	<i>ty<sub>3</sub>-ly<sub>5</sub></i>	139	1	22		<i>th<sub>1</sub>-S<sub>h</sub></i>	41	0	3	
	<i>ty<sub>3</sub>-met<sub>13</sub></i>	53	1	26		<i>S<sub>h</sub>-py<sub>2</sub></i>	44	0	9	
	<i>met<sub>13</sub>-ac<sub>2</sub><sup>r</sup></i>	50	1	29		<i>th<sub>1</sub>-py<sub>2</sub></i>	60	0	18	
	<i>ly<sub>5</sub>-ac<sub>2</sub><sup>r</sup></i>	13	2	31	Frag. 7	<i>SU<sub>3</sub>-MA<sub>3</sub></i>	124	0	0	
	<i>ac<sub>2</sub><sup>r</sup>-tr<sub>5</sub></i>	44	5	111		<i>MA<sub>3</sub>-MG<sub>2</sub></i>	123	0	0	
	<i>tr<sub>5</sub>-ac<sub>3</sub><sup>r</sup></i>	42	0	22		<i>SU<sub>3</sub>-MG<sub>2</sub></i>	61	0	1	
	<i>ac<sub>3</sub><sup>r</sup>-le<sub>1</sub></i>	53	0	12	Frag. 8	<i>ga<sub>5</sub>-th<sub>2</sub></i>	53	0	8	
	<i>tr<sub>5</sub>-le<sub>1</sub></i>	365	0	132						

PD=parental ditype ascus; NPD=nonparental ditype; T=tetatype.

contributing to our difficulties. First, the chromosome number is higher than that anticipated from the early cytological studies where the estimates for the haploid number n ranged from 1 to 4 (DELAMATER 1950; McCLARY, WILLIAMS, LINDEGREN, and OGUR 1957; GANESAN 1959). The linkage studies, based on independently segregating centromere markers, provide evidence for 14 chromo-

TABLE 3

Ascus-type ratios (PD:NPD:T) for new centromere markers in combination with centromere markers on the established linkage groups

	I <i>ad</i> <sub>1</sub>	II <i>ga</i> <sub>1</sub>	III <i>a/a</i>	IV <i>tr</i> <sub>1</sub>	V <i>ur</i> <sub>3</sub>	VI <i>hit</i> <sub>2</sub>	VII <i>le</i> <sub>1</sub>	VIII <i>ar</i> <sub>4</sub>	IX <i>hi</i> <sub>6</sub>																		
<i>met</i> <sub>3</sub>	35	42	33	11	8	10	13	9	16	14	5	12	34	44	36	41	33	38									
<i>met</i> <sub>14</sub>	16	31	18	63	63	6	65	53	29	17	16	21	21	29	4	15	7	15	34	39	44						
<i>thr</i> <sub>5</sub>	39	27	32	18	14	19	43	36	75	52	52	45	15	28	25	29	26	53	24	27	26	45	34	68	32	33	46
<i>ly</i> <sub>7</sub>	71	84	166	125	130	271	120	134	331	153	147	299	149	146	328	55	67	174	143	132	276	55	57	146	88	67	204
<i>p</i> <sub>8</sub>	17	16	6	70	52	18	55	50	58	102	79	2	58	47	22	13	7	6	43	37	6	18	14	13	68	69	70

TABLE 4

Ascus-type ratios (PD:NPD:T) between the new centromere markers

	<i>met</i> <sub>3</sub>	<i>met</i> <sub>14</sub>	<i>thr</i> <sub>5</sub>	<i>ly</i> <sub>7</sub>
<i>met</i> <sub>14</sub>	15:17:5*	.....	.....	.....
<i>thr</i> <sub>5</sub>	3:4:9	6:10:9	.....	.....
<i>ly</i> <sub>7</sub>	11:14:25	22:29:44	26:23:58	.....
<i>p</i> <sub>8</sub>	60:57:7	51:49:8	12:17:16	15:12:18

\* The centromere of linkage group X was marked by *is*<sub>3</sub> for the tetrad analysis with *met*<sub>14</sub>.



100 centimorgans for several of the chromosome arms. In fact, it would appear that the average length of the chromosome arms might be at least 100 centimorgans. This estimate is based on the fraction of genes showing centromere linkage, 34/102, and the assumptions: (1) that there is an even distribution of markers along the chromosome arm, and (2) that genes less than 33 units from the centromere have been recognized as centromere markers. There are two opposing biases in the above sampling of centromere markers: (1) some centromere-linked genes may have been missed. For example, genes like *pha*<sub>2</sub> and *SU*<sub>2</sub> which show more than 60% but less than 67% second-division segregation were not included among the centromere markers unless they had been mapped on the established chromosomes. (2) The 102 loci for which second-division segregation frequencies have been presented in Table 1 include more than a random sample of centromere-linked genes. More than 150 markers were given a rough screening for centromere linkage—usually about 20 asci were analyzed—and generally only those genes which gave a second-division segregation frequency of less than 50% were pursued further unless they were involved in a biosynthetic pathway of interest to our colleagues. When both factors are considered, 100 centimorgans is a conservative estimate of the average chromosome arm length.

From the above estimate for the chromosome arm length, and assuming that the chromosomes are metacentric, we could expect a total map length as great as 3600 centimorgans. A summation of the gene-to-gene and gene-to-centromere intervals of the maps in Figure 1 gives a minimum length of 1300 centimorgans. Thus, less than half of the predicted genome is encompassed in these maps.

#### SUMMARY

In a search for centromere-linked genes, tetrad analyses have been made to obtain second-division segregation frequencies for 102 loci. Thirty-four of the genes were shown to be centromere-linked and located on 14 different chromosomes. Twenty-four other genes have been mapped in the linkage groups, and another 28 genes comprise eight linkage groups not yet associated with a centromere. The length of the genetic map now exceeds 1300 centimorgans, but it is estimated that the present map covers less than half of the entire genome.

#### LITERATURE CITED

- Carbondale Yeast Genetics Conference, 1963 *Microbial Genet. Bull.* **19** (Suppl.).
- DELAMATER, E. D., 1950 The nuclear cytology of vegetative diplophase of *Saccharomyces cerevisiae*. *J. Bacteriol.* **60**: 321-332.
- DESBOROUGH, S., E. E. SHULT, T. YOSHIDA, and C. C. LINDEGREN, 1960 Interference patterns in family Y-1 of *Saccharomyces*. *Genetics* **45**: 1467-1480.
- DOUGLAS, H. C., and D. C. HAWTHORNE, 1964 Enzymatic expression and genetic linkage of genes controlling galactose utilization in *Saccharomyces*. *Genetics* **49**: 837-844.
- GANESAN, A. T., 1959 The cytology of *Saccharomyces*. *Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol.* **31**: 149-174.

- HAWTHORNE, D. C., and R. K. MORTIMER, 1960 Chromosome mapping in *Saccharomyces*: Centromere-linked genes. *Genetics* **45**: 1085-1110. — 1963 Super-suppressors in yeast. *Genetics* **48**: 617-620.
- HOWE, H. B., 1956 Crossing over and nuclear passing in *Neurospora crassa*. *Genetics* **41**: 610-622.
- HWANG, Y. L., G. LINDEGREN, and C. C. LINDEGREN, 1963 Mapping of the eleventh centromere in *Saccharomyces*. *Can. J. Genet. Cytol.* **5**: 290-298. — 1964 The twelfth chromosome of *Saccharomyces*. *Can. J. Genet. Cytol.* **6**: 373-380.
- JAMES, A. P., and B. LEE-WHITING, 1955 Radiation-induced genetic segregations in vegetative cells of yeast. *Genetics* **40**: 826-831.
- LINDEGREN, C. C., and G. LINDEGREN, 1951 Linkage relationships in *Saccharomyces* of genes controlling the fermentation of carbohydrates and synthesis of vitamins, amino acids and nucleic acid components. *Indian Phytopathol.* **4**: 11-20.
- LINDEGREN, C. C., G. LINDEGREN, E. SHULT, and Y. L. HWANG, 1962 Centromeres, sites of affinity and gene loci on the chromosomes of *Saccharomyces*. *Nature* **194**: 260-265.
- MCCCLARY, D. O., M. A. WILLIAMS, C. C. LINDEGREN, and M. OGUR, 1957 Chromosome counts in a polyploid series of *Saccharomyces*. *J. Bacteriol.* **73**: 360-364.
- PERKINS, D. D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. *Genetics* **34**: 607-626.
- PONTECORVO, G., and E. KÄFER, 1958 Genetic analysis based on mitotic recombination. *Advan. Genet.* **9**: 71-104.
- ROMAN, H., 1956 A system selective for mutations affecting the synthesis of adenine in yeast. *Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol.* **26**: 299-314.
- TAMAKI, S., 1965 Chromosome behavior at meiosis in *Saccharomyces*. *J. Gen. Microbiol.*, in press.