GENETIC MAPPING IN SACCHAROMYCES

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IN 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 μg/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 KÄFER 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 ⅔ 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 ⅔ for genes considerably removed from the centromere.

Our first map included the following centromere markers: I–ad_1, II–ga_1, III–α and hi_4, IV–tr_1 and ga_3, V–ur_3, VI–hi_2, VII–le_1, tr_5, and ad_6, VIII–p_1, ar_4, and thr_1, IX–hi_6 and ly_1, and X–met_2. However, we concur in the assessment by LINDEGREN *et al.* (1962) that the use of met_2 as a centromere marker defining a new linkage group was premature. In its stead, they proposed that another methionine gene, met_3, be adopted as the centromere marker of chromosome X (LINDEGREN *et al.* 1962). HWANG *et al.* (1963) showed that met_{14} 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_5 and ly_7. Our own studies of hybrids heterozygous for thr_5 and ly_7 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_5 for linkage group XII and have assigned ly_7 to the centromere of linkage group XIII. Another new linkage group, XIV, is established with the demonstration that p_8 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_1^r (DESBOROUGH, SHULT, YOSHIDA, and LINDEGREN 1960), p_9, ga_7, and ga_{10} (DOUGLAS and HAWTHORNE 1964); III, le_2 (LINDEGREN *et al.* 1962);

TABLE 1

Second-division segregation frequencies of 102 genes in Saccharomyces

Gene	Division segregation First	Second	% second	Gene	Division segregation First	Second	% second	Gene	Division segregation First	Second	% second
α	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	$S\text{-}ser_1$	39	43	52.5
ad_4	47	94	66.7	ly_9	33	28	46.8	$s\text{-}met_1$	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/α* 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_1 and thr_4) or threonine plus methionine (thr_2, thr_3, thr_5, and thr_6), *tr-* tryptophan, *ty-* tyrosine (ty_1), tyrosine plus phenylalanine ($ty_{2,4}$) or tyrosine plus phenylalanine plus tryptophan (ty_3), 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_a to S_h- super suppressors, (HAWTHORNE and MORTIMER 1963), *S-ser*$_4$ and *s-met*$_4$- specific suppressors. The subscripts (number or letter) identify particular genetic loci.

V, *S–ser*$_1$ and ar_9 (Table 2); VI, met_{10} (LINDEGREN *et al.* 1962) and S_d (Table 2); VII, ac_3^r (Table 2); X, is_3 (Table 2); XII, ROC_1^r (Table 2); and XIV, ly_9 (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 α, 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^r_2–ly_5–$ad_{5,7}$, c–ty_2–thr_2–tr_4–ad_8, and c–ad_2–ser_1–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_5–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_3–MA_3–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	p_9-ac_1^r	32	0	19		le_1-ad_6	208	9	290
	p_9-ga_1	72	0	70		ad_6-MA_1	14	15	49
	ac_1^r-ga_1	93	1	52		MA_1-SU_1	108	0	0
	ga_1-ga_7	313	0	0		MA_1-ad_3	42	7	89
	ga_1-ga_{10}	59	0	0	VIII	p_1-ar_4	371	0	30
	ga_7-ga_{10}	72	0	0		ar_4-thr_1	453	0	138
	ga_1-ly_2	101	23	259		thr_1-CU_1	259	2	225
	ly_2-ty_1	129	6	200		CU_1-p_3	88	4	148
	p_{11}-ty_1	17	0	0	IX	hi_6-ly_1	138	17	256
	ty_1-met_8	87	0	42	X	is_3-met_3	69	0	5
	met_8-hi_7	53	0	35	XII	thr_5-ROC_1^r	53	0	5
	ty_1-hi_7	34	3	90		thr_5-ga_2	32	0	31
III	hi_4-le_2	353	3	165	XIV	p_8-ly_9	24	2	25
	le_2-α	209	12	260	Frag. 1	hi_8-ser_1	249	0	68
	α-thr_4	268	4	162		hi_8-ad_9	16	0	5
	thr_4-MA_2	137	4	145		ad_9-ad_2	23	0	16
IV	ga_3-tr_1	123	0	1		ser_1-ad_2	100	1	109
	tr_1-s-met_1	17	1	32		hi_8-ad_2	90	4	198
V	S-ser_1-ur_3	41	2	41		ad_2-ac_4^r	107	5	124
	ur_3-ar_9	52	0	9	Frag. 2	ty_2-thr_2	329	0	90
	ar_9-thr_3	14	1	21		thr_2-S_f	46	0	4
	ur_3-thr_3	73	2	131		ty_2-S_f	36	0	22
	thr_3-hi_1	38	0	3		thr_2-tr_4	18	11	52
	hi_1-ar_6	63	0	9		tr_4-ad_8	32	0	46
	hi_1-is_1	49	0	20	Frag. 3	pha_2-met_2	60	1	87
	is_1-tr_2	56	0	13		met_2-p_2	102	0	33
	ar_6-tr_2	27	0	14	Frag. 4	SU_2-hi_5	11	1	19
VI	S_d-hi_2	65	1	41		hi_5-ly_{11}	24	0	14
	hi_2-met_{10}	77	0	49	Frag. 5	MA_4-met_1	40	3	37
VII	$ad_{5,7}$-ly_5	28	9	59		met_1-ur_1	53	55	201
	$ad_{5,7}$-ty_3	39	17	110		ur_1-tr_3	42	0	5
	ty_3-ly_5	139	1	22	Frag. 6	th_1-S_h	41	0	3
	ty_3-met_{13}	53	1	26		S_h-py_2	44	0	9
	met_{13}-ac_2^r	50	1	29		th_1-py_2	60	0	18
	ly_5-ac_2^r	13	2	31	Frag. 7	SU_3-MA_3	124	0	0
	ac_2^r-tr_5	44	5	111		MA_3-MG_2	123	0	0
	tr_5-ac_3^r	42	0	22		SU_3-MG_2	61	0	1
	ac_3^r-le_1	53	0	12	Frag. 8	ga_5-th_2	53	0	8
	tr_5-le_1	365	0	132					

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

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 *ad*$_1$			II *ga*$_1$			III *a/α*			IV *tr*$_1$			V *ur*$_3$			VI *hi*$_2$			VII *le*$_1$			VIII *ar*$_4$			IX *hi*$_8$		
met$_3$	35	29	19	45	42	33	34	27	48	11	8	10	8	11	13	13	9	16	14	5	12	34	44	36	41	33	38
met$_{14}$	16	31	7	39	34	18	54	32	41	63	63	6	65	53	29	17	16	21	21	29	4	15	7	15	34	39	44
thr$_5$	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
ly$_7$	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
p$_8$	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

	met$_3$	*met*$_{14}$	*thr*$_5$	*ly*$_7$
met$_{14}$	15:17: 5*	.....	.....	.....
thr$_5$	3: 4: 9	6:10: 9	.....	.....
ly$_7$	11:14:25	22:29:44	26:23:58	.....
p$_8$	60:57: 7	51:49: 8	12:17:16	15:12:18

* The centromere of linkage group X was marked by *is*$_3$ for the tetrad analysis with *met*$_{14}$.

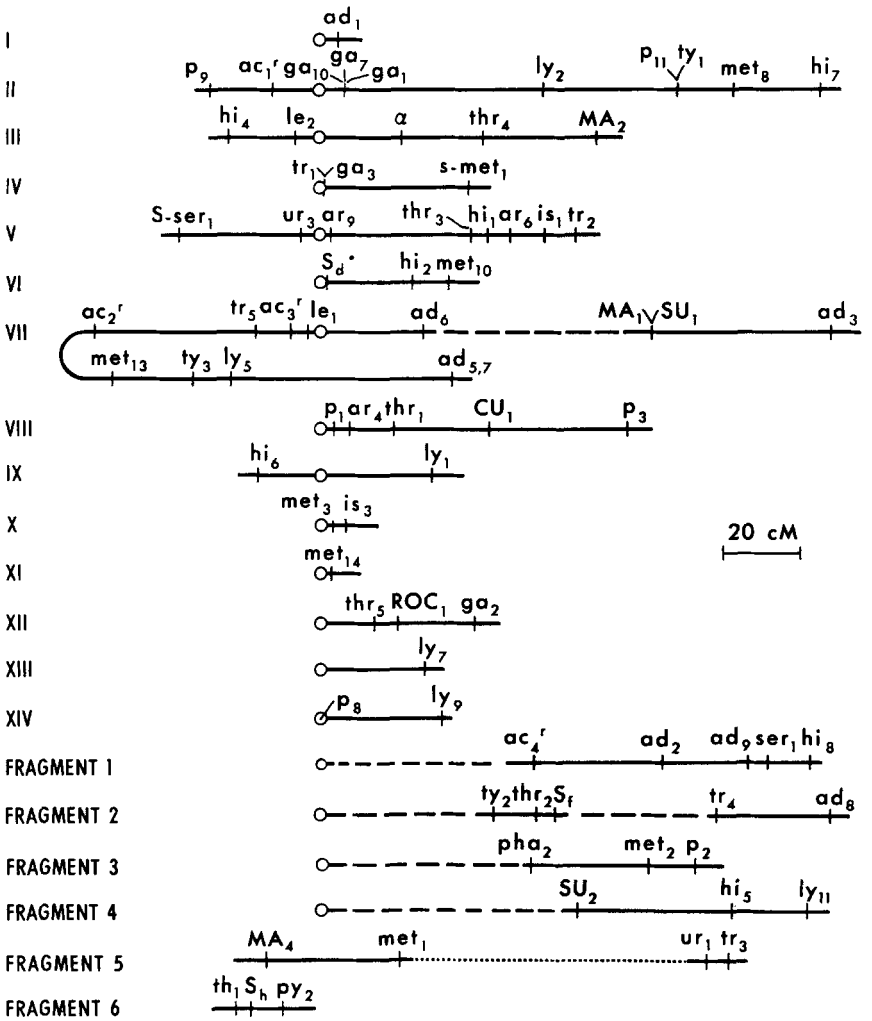

FIGURE 1.—Genetic maps of Saccharomyces. Linkage established by tetrad analysis is represented by a solid line. The map distances were computed from the data in Tables 1 and 2. For the linkages established by mitotic segregation (dashed line) or by trisomic analysis (dotted line) only the sequences have significance.

somes. However, the distribution of the 34 centromere-linked genes on these 14 chromosomes—one chromosome with 5 genes, one with 4, four with 3, five with 2, and three with 1—leads to an estimate of 16 or 17 for n if a Poisson distribution is assumed for this sample. This estimate for n has received support from the recent cytological studies of S. TAMAKI (1965) who has counted 18 bivalents in the first meiotic prophase of diploid hybrids from our breeding stocks.

Without additional pertinent mutants for study, analysis beyond this point is

Secondly, the suspicion that we might be dealing with genetically long chromosomes has also been upheld by the demonstration of genetic lengths exceeding

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_2 and SU_2 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.

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