X-RAY AND MEIOTIC FINE STRUCTURE MAPPING OF THE ADENINE-8 LOCUS IN *SACCHAROMYCES CEREVISIAE*[1]

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WHILE it has been demonstrated in a variety of organisms that genes differ in their spontaneous mutation rates, little is known concerning the basis of these differences. Since the spontaneous mutations affecting adenine biosynthesis in *Saccharomyces cerevisiae* can be obtained by a selective technique (ROMAN 1956), they provide a convenient system in which to study the factors involved in spontaneous mutation rates. In this yeast there are two adenine requiring mutants, adenine-1 and adenine-2, which produce a red pigment as a result of the block in adenine synthesis. Haploid cultures of either adenine-1 or adenine-2 accumulate white and pink mutants which outgrow the parental red strains. Genetic analysis of these color variants has shown that the interference with pigment production is most often the result of a second mutation in the adenine pathway.

The mutants thus obtained are unequally distributed among six loci (ROMAN 1956; JONES 1964). The question of this differential mutability at the adenine loci has been examined by JONES (1964) in a comparative study of the adenine-3 and adenine-6 loci. The results of her study indicated that there was a positive correlation between the size of these loci, measured by their recombinational lengths and numbers of mutable sites, and the frequency with which they give rise to spontaneous mutations. The twofold difference in the size of these loci, however, was not sufficient to explain the five to tenfold higher mutability of adenine-6 as compared with adenine-3. Further study of the properties of the mutants of each of these loci led to the suggestion that this discrepancy might be due to the failure to recover a substantial fraction of adenine-3 mutants, i.e., that the mutation frequency of adenine-3 is actually higher than is observed.

The adenine-8 locus was chosen for investigation in this study because mutants of this gene occur with approximately the same frequency as those of the adenine-3 locus but their properties do not suggest that a large number of mutations are not being recovered. Accordingly adenine-8 should be smaller than adenine-3 and adenine-6, in terms of both recombinational length and number of mutable sites, if gene size is an important component of mutation frequency.

In this study the recombinational length of the adenine-8 locus was estimated by two independent methods of allelic mapping. A meiotic fine structure map was constructed using allelic recombination frequencies after meiosis as a meas-

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ure of the distance between mutant sites and an X-ray map was constructed from X-ray induced mitotic allelic recombination rates. The results are described below.

MATERIALS AND METHODS

Yeast strains: The strains of *Saccharomyces cerevisiae* employed in this study were obtained from DR. B. DORFMAN, DR. D. C. HAWTHORNE, and DR. E. W. JONES.

Media: The amounts of the various ingredients indicated are those required for the preparation of one liter of medium. *Synthetic complete*—20 g dextrose, 6.7 g Difco Yeast Nitrogen Base, 10 mg adenine, 10 mg uracil, 10 mg histidine, 60 mg isoleucine, 60 mg leucine, 20 mg lysine, 10 mg methionine, 10 mg tryptophan, 10 mg arginine, 15 g agar; *Supplemented Yeast Extract Peptone* (YEP)—20 g dextrose, 20 g Bacto-Peptone, 10 g Yeast Extract, 20 mg adenine, 20 mg uracil, 40 mg lysine, 20 mg tryptophan; *Sporulation medium*—1 g dextrose, 2.5 g yeast extract, 10 g potassium acetate, 20 mg adenine, 20 mg uracil, 40 mg lysine, 20 mg tryptophan; *Osmotic medium*—Osmotic media were media to which various amounts of KCl were added to raise the osmotic pressure. Three concentrations of KCl were used: 0.5 M, 1.0 M, and 2.0 M.

X-ray irradiation procedure: 44 hr cultures grown at 30°C in supplemented YEP medium were irradiated on the surface of solid medium in open plastic Petri dishes. The source of X rays was a Machlett OEG-60 beryllium window tube operated at 50 KVP and 20 MA from a Picker Control Rectifier unit. The dose rate in air at the level of the plates was 72 roentgens per second as estimated from calibration of the tube with a Victoreen r-meter and a nylon wall chamber. The cells were washed once in sterile distilled water prior to irradiation. Between 10^6 and 10^8 cells, the density depending upon the cross, were spread on the surface of adenineless medium. Plates were irradiated at six different doses: 3, 5, 10, 15, 20, and 25 seconds of X rays. The lowest dose, 3 seconds, was used when a large response to X rays could be anticipated.

Ultraviolet irradiation procedure: All irradiations were performed using a Hanovia-Vycor lamp (95% 2537 Angstroms wave length). The dose rate at the level of the material to be irradiated was 1600 ergs/cm^2/second.

Procedure for sporulation: Diploids for sporulation were grown 48 hrs at 30°C in 5 ml of supplemented YEP medium. The cells were washed once in sterile distilled water and suspended in sporulation medium at 5×10^7 cells per ml. Maximum sporulation (40–60%) was obtained after five days of aerobic incubation at 30°C.

Tests for response to supersuppressors: Approximately 6×10^8 cells of each haploid to be tested were distributed on adenineless medium or adenineless tryptophanless medium. Half of the plates were irradiated with ultraviolet light for two minutes to increase the frequency of supersuppressor mutations. The plates were then incubated at 30°C and examined at intervals to the 14th day for the appearance of prototrophs. As colonies appeared they were purified by restreaking and tested for their ability to grow on adenineless or adenineless tryptophanless medium.

EXPERIMENTAL RESULTS

Spectrum of spontaneous adenine mutations: The spontaneous *ad-8* mutations employed in this study were obtained in an *ad-2* strain (C-62-30B) by the selective system reported by ROMAN (1956). When an *ad-1* or *ad-2* strain is grown on solid YEP medium the double mutants appear as white and pink papillae on the surface of an otherwise red colony. For this study, cells of the C-62-30B strain were spread on solid YEP medium and incubated at 30°C for seven days. After this time one papilla was picked from each colony and purified by restreaking on YEP medium. The mutants were then classified by allelic testing.

The spectrum of spontaneous mutants obtained is shown in Table 1. As ob-

TABLE 1

Relative frequencies of spontaneous adenine mutations obtained in strain C-62–30B

	ad-3	*ad-4*	*ad-5*	*ad-6*	*ad-7*	*ad-5–7*	*ad-8*	Total
No.	106	133	144	592	150	147	98	1370
%	7.7	9.7	10.5	43.2	10.9	10.7	7.1	

served by ROMAN (1956) and JONES (1964), mutants of the *ad-6* gene are the most frequently recovered while mutants of *ad-3* and *ad-8* are among the less frequently obtained mutations.

Physiological characterization of the ad-8 *mutants:* Eleven of the ninety-eight *ad-2 ad-8* double mutants isolated, slowly turned red on solid YEP medium at 30°C. These mutants will be referred to as "leaky." The leaky phenotype suggests that the *ad-8* product is present in altered form, or in reduced amount. The remainder of the double mutants formed white colonies on YEP medium at 30°C. The *ad-2 ad-8* double mutants were tested for response to changes in temperature and osmotic pressure and suppression by supersuppressors (HAWTHORNE and MORTIMER 1963).

1. *Temperature sensitivity and osmotic remediability:* Auxotrophic mutants of yeast which exhibit growth on minimal medium in response to temperature changes, increased osmotic pressure, or a combination of these, have been interpreted to be missense mutations (HAWTHORNE and FRIIS 1964; MANNEY 1964). To determine the response of the *ad-8* mutants in the *ad-2 ad-8* genotype to changes in temperature and osmotic pressure it was necessary to rely on pigment production. The *ad-2* allele (*ad-2–1*) of the double mutants is neither temperature sensitive nor osmotic remedial; thus strains carrying only this mutation (e.g. C-62-30B), form the red pigment at the temperatures and osmotic pressures tested. Hence the appearance of red pigment in a non-leaky *ad-2 ad-8* double mutant may be interpreted as having resulted from a restoration of the *ad-8* function in response to the environmental change. The mutants were seeded on synthetic complete medium and on solid YEP medium containing KCl (0.5, 1.0 and 2.0 M/liter) to raise the osmotic pressure. Plates of each type were incubated at four different temperatures (18, 25, 30, and 33°C) and examined at intervals for 20 days for the appearance of color. The results of the tests are summarized in Table 2. Five of the ninety-eight mutants formed pigment in response to either temperature change or osmotic pressure. The eleven mutants originally classified as leaky slowly turned red under all test conditions. Since the leaky phenotype suggests that the *ad-8* product is formed, it is likely that these mutants are also missense mutations.

2. *Response to supersuppressors:* Supersuppressors are allele specific suppressors that are capable of restoring the wild-type phenotype, in whole or in part, of a variety of unrelated auxotrophic mutants of yeast. Recent studies suggest that supersuppressible mutations are nonsense mutants (HAWTHORNE and MORTIMER 1963; MANNEY 1964; MORTIMER and HAWTHORNE 1966).

TABLE 2

Properties of 98 spontaneous adenine-8 *mutants*

Class	Number in class	Interpretation
Supersuppressible	24	nonsense mutants
Temperature sens.	5	missense mutants
Osmotic remedial	3*	missense mutants
Leaky	11	missense mutants
Remainder	58	?
Total	98	

* Also temperature sensitive.

In order to estimate the number of supersuppressible alleles among the *ad-8* mutants, advantage was taken of the fact that the allele of *ad-2* carried by the double mutants responds to a wide range of supersuppressors (HAWTHORNE and MORTIMER 1963, HAWTHORNE, personal communication). To determine whether a particular *ad-8* mutant was supersuppressible the *ad-2 ad-8* double mutants were plated on adenineless medium. If the *ad-8* mutation is supersuppressible a single suppressor of both *ad-2–1* and the *ad-8* allele in question could restore the ability of the double mutant to grow on adenineless medium. However, it is extremely unlikely that a strain carrying a non-supersuppressible *ad-8* mutant would give adenine independent prototrophs since this would require the occurrence of two mutational events in the same cell: back mutation at the *ad-8* locus and a mutation restoring *ad-2* function.

Twenty-four of the eighty-seven double mutants tested were found to be supersuppressible (Table 2) by this technique. Fifty-eight mutants did not exhibit supersuppressibility and also were not temperature sensitive, osmotically remediable or leaky. A sample of eleven of these mutants was obtained free of the *ad-2* mutation and was tested for reversion. Nine of the eleven reverted spontaneously. Thus it appears likely that this class of mutants consists primarily of base pair substitutions, either missense or nonsense.

3. *Properties of the* ad-8 *mutants isolated for fine structure mapping:* 22 of the *ad-8* mutants were chosen at random for fine-structure mapping to obtain an estimate of the size of the locus and to compare the distributions of the various classes of mutants on the fine structure map of the gene. The *ad-2 ad-8* mutants were crossed to an adenine independent strain of opposite mating type. From these diploids *a* and α haploid derivatives were obtained after sporulation, free of the *ad-2* mutation, and in genotypes suitable for intercrossing.

The *ad-8* single mutants isolated were tested again for osmotic remediability, temperature sensitivity, leakiness, and response to supersuppressors. It was now possible to use growth on adenineless medium as an index of response to changes in temperature and osmotic pressure.

The results of these tests are summarized in Table 3. The mutants which were leaky or temperature sensitive by the criterion of pigment formation in the *ad-2 ad-8* genotype gave positive results by the growth tests as well. One mutant,

TABLE 3

Properties of 22 adenine-8 *mutants isolated for fine structure mapping*

Class	Number in class	Alleles in class
Reverting mutants		
supersuppressible	9	*ad-8–1*, *8–2*, *8–3*, *8–4*, *8–6*, *8–8*, *8–10*, *8–14*, *8–19*
temperature sensitive	2	*ad-8–17*, *8–20*
leaky	3	*ad-8–3*, *8–17*, *8–20*
osmotic remedial	0	
others	9	*ad-8–5*, *8–7*, *8–9*, *8–11*, *8–13*, *8–15*, *8–16*, *8–21*, *8–22*
Non-reverting mutants	2	*ad-8–12*, *8–18*

ad-8–3, which was previously classified as a non-leaky supersuppressible mutant, grew slowly on adenineless medium at all temperatures and osmotic pressures tested, and was therefore classified as a leaky mutant.

To test the single mutants for supersuppressibility the strains were plated on synthetic medium lacking adenine and tryptophan. All of the cultures carried a supersuppressible tryptophan mutation, *try-5–2*. This mutant, like *ad-2–1*, responds to a broad spectrum of supersuppressors (HAWTHORNE and MORTIMER 1963; HAWTHORNE, personal communication). The appearance of prototrophs on the selective medium indicated that the adenine and tryptophan requirements were both suppressed.

All of the mutants which responded to supersuppressors of *ad-2–1* also responded to supersuppressors of *try-5–2* (Table 3). Two alleles, *ad-8–1* and *ad-8–10*, which were classified as non-supersuppressible mutants by the previous test also responded. This result was not unexpected since the *try-5–2* allele is suppressed by some supersuppressors that do not act on the *ad-2–1* allele (HAWTHORNE, personal communication).

The 22 *ad-8* mutants were tested for spontaneous reversion. 20 of the mutants reverted spontaneously. Two of the mutants *ad-8–12* and *ad-8–18*, failed to revert. In subsequent experiments more than 10^9 cells of each have been plated on adenine deficient medium without observing revertants of either allele.

Most of the reversion frequencies ranged from 1 to 200 prototrophs per 10^8 cells plated. Two of the mutants, *ad-8–7* and *ad-8–11*, gave reversion frequencies approximately one hundred times greater. Ten cultures of each mutant grown from small inocula (ca 10^3 cells) were sampled for the frequency of adenine independent mutants at stationary phase. The average frequency of prototrophs in the *ad-8–7* cultures was $44/10^6$ cells plated and in the *ad-8–11* cultures, $43/10^6$ cells plated.

Meiotic and X-ray mapping of the ad-8 *mutants:* The meiotic fine structure map was constructed using the frequency of adenine independent prototrophs among whole asci plated on adenineless medium as a measure of the recombination distance between the mutant sites. Diploid cultures were grown from small inocula, an aliquot was plated on adenineless medium to assay the frequency of prototrophs produced during mitotic growth, and a portion of each culture was then sporulated.

The average diploid gave forty to sixty percent asci at the completion of sporulation. To estimate the frequency of allelic recombinants after meiosis, the mixture of asci and unsporulated cells was plated on adenineless medium to assay the frequency of allelic recombinants in the total population. Since it is known that the allelic recombinants produced in sporulation medium are largely restricted to the cells which form asci (SHERMAN and ROMAN 1963; ESPOSITO and ROMAN, unpublished), the percentage of asci in each culture was then determined by haemocytometer counts and the frequency of adenine independent prototrophs observed was then corrected to the frequency among asci, using the formula shown below:

$$\text{prototrophs}/10^5 \text{ asci} = \frac{\text{prototrophs}/10^5 \text{ plating units after sporulation} - \text{prototrophs}/10^5 \text{ diploid cells before sporulation}}{\text{frequency of asci}}$$

Meiotic heteroallelic recombination frequencies of all pairwise crosses are shown in Figure 1. The values are expressed as the number of prototrophs per 10^5 asci. Of 231 heteroallelic crosses, 10 failed to recombine.

The mitotic X-ray map was constructed by the method reported by MANNEY and MORTIMER (1963). When a heteroallelic diploid is irradiated with sublethal doses of X rays, wild-type revertants increase linearly with dose at a rate which depends upon the particular pair of alleles in the cross. These authors have shown that it is possible to order mutant sites within a gene using the slope of the dose-effect curves of heteroallelic diploids as a measure of the distance between the sites.

In the mapping of the *ad-8* alleles both the homoallelic and heteroallelic diploids were irradiated with five doses of X rays to 1800 roentgens. The slopes of the X-ray curves obtained are given in Figure 2. The slopes of the response curves of the homoallelic diploids are also presented; the majority of them are 5–10 times

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0	286	1762	360	240	504	859	159	0	528	1973	1426	0	5	189	91	51	12	19	777	164	375
2		0	2408	25	0	1354	75	686	447	6	75	1486	194	156	0	40	122	1274	213	1193	87	17
3			0	2782	3288	406	4792	284	1810	485	2401	1099	2465	1132	1588	1782	1414	3617	1210	295	2141	179
4				0	23	1318	58	883	243	9	173	1460	300	223	27	93	83	379	245	1517	2448	34
5					0	1198	50	454	195	9	50	1083	303	138	0	3	149	1439	161	896	49	0
6						0	890	121	864	503	1185	0	731	306	753	1026	435	1809	502	5	1076	1242
7							0	923	997	30	69	1263	634	976	37	756	767	2160	741	2393	635	69
8								0	297	320	821	678	374	191	526	245	181	732	192	162	244	577
9									0	196	906	1820	0	4	247	214	79	44	50	2474	127	178
10										0	227	848	119	283	5	70	161	1838	238	966	67	28
11											0	1752	562	414	67	68	482	609	260	1440	173	106
12												0	1123	1211	1525	1463	1790	2060	1346	0	1721	1830
13													0	54	48	32	35	1376	103	1027	4	60
14														0	122	170	46	0	7	645	98	153
15															0	38	132	1065	190	1046	49	0
16																0	63	1256	47	440	22	39
17																	0	336	8	611	35	92
18																		0	144	179	673	849
19																			0	544	63	117
20																				0	784	1154
21																					0	71
22																						0

FIGURE 1.—Meiotic heteroallelic recombination frequencies/10^5 asci and homoallelic reversion frequencies/10^5 asci of crosses involving twenty-two *adenine-8* mutants.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	.000	1.00	2.56	.972	.625	983	3.05	.388	.003	.805	1.33	2.61	.003	.744	.561	.836	.186	.009	.097	.972	.611	.611
2		.008	2.96	.069	.127	2.01	.794	1.33	1.28	.090	.569	1.75	1.66	1.16	.008	.136	.363	1.11	.277	2.61	.150	.008
3			.002	3.97	2.63	.822	4.30	2.08	5.55	3.47	3.83	.311	4.11	3.38	2.16	3.05	2.44	3.75	1.21	.750	2.16	2.32
4				.010	.097	2.15		1.91	1.20	.100		5.55	5.17	1.05	.133	.400	1.22	3.47	.660	4.00	.638	.160
5					.004	1.94		1.26	.560	.038	.483		3.16	1.16	.008	.236	.47	1.11	.305	3.13	.277	.013
6						.002	3.69	.750	6.66	3.44	5.38	.001		1.58	1.77	1.94	1.55	3.00	1.00	.050	2.77	2.08
7							.000	3.72	4.05	.527	2.11	4.05	3.16	2.88	.116	4.50	3.05	3.16	3.33		2.58	
8								.008	7.27	3.25	2.16	1.73	3.33	.610	.610	3.22	1.66	1.66	.470	.916	1.50	.555
9									.004	3.27	2.27	2.77	.002	.040	1.16	3.47	2.22	.094	.694	5.77	1.83	2.36
10										.000	1.44	2.77	7.15	1.22	.050	.566	.923	6.66	.722	3.88	.660	.145
11											.070			2.0					1.02	6.53		
12												.000	7.61	7.33	3.61	4.27	1.72	.810	1.44	.004	3.94	2.27
13													.002	.708	.383	.322	.302	3.61	.812	3.22	.110	.266
14														.018	.090	1.61	.211	.172	.233	1.37	.444	.444
15															.009	.27	2.02	1.80	6.33	3.54	.294	.007
16																.001	.347	.944	.847		.205	
17																	.037	1.11	.200		.511	.633
18																		.000	.250	3.16	.266	1.55
19																			.016	.777	.472	.555
20																			.05	2.61	2.31	
21																				.029	.400	
22																					.011	

FIGURE 2.—X-ray induced mitotic heteroallelic recombination rates and homoallelic reversion rates/10^8 cells/roentgen of crosses involving twenty-two *adenine-8* mutants.

lower than the lowest response of the heteroallelic diploids. X-ray induced recombination could not be measured in thirteen crosses involving *ad-8–7* and *ad-8–11* because of the high spontaneous reversion frequencies of these mutants.

In eleven of the heteroallelic crosses the response to irradiation could not be distinguished from that of one or both of the corresponding homoallelic diploids. Most of the mutant pairs which did not recombine following X-ray treatment were those which did not recombine during meiosis.

Meiotic and X-ray maps of the ad-8 *mutants:* The meiotic and X-ray fine structure maps of the 22 *ad-8* mutants are shown, drawn to scale, in Figure 3. On these

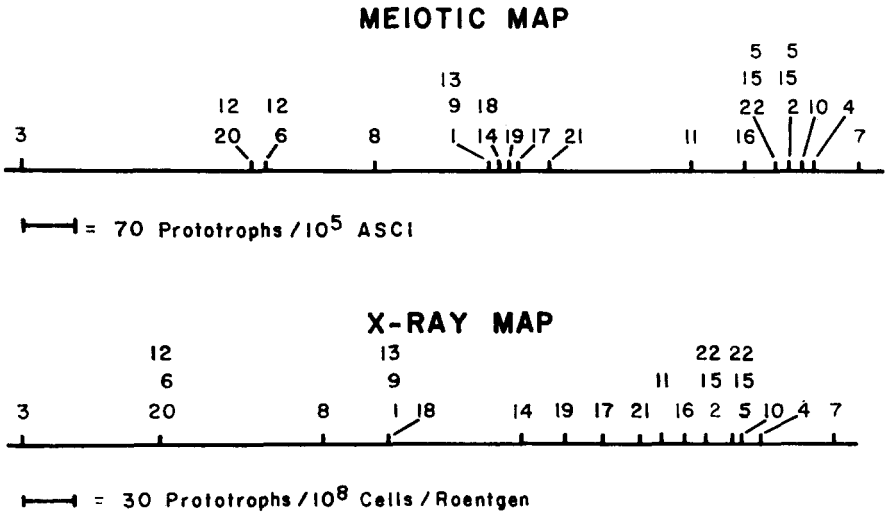

FIGURE 3.—Meiotic and X-ray recombination maps of the *adenine-8* mutants. Mutants shown directly above one another at the same site are those which were not separable by recombination.

maps the mutants which were not separable by recombination are shown directly above one another at the same site. The sequence of mutants which recombined following both X-ray irradiation and meiosis is the same on both maps. The few discrepancies in sequence involve the positions of mutants which were separable by recombination by one method but not by the other.

While the order of the mutants separated by meiosis and X rays is the same on the two maps, the mutants appear to be more evenly distributed on the X-ray map. This is due primarily to the fact that the length of the interval from *ad-8*–1 to *ad-8*–21 represents approximately one third (1.65/4.64) of the total length of the X-ray map but less than one tenth (83/1033) of the length of the meiotic map. The mutants on both sides of this segment are distributed similarily relative to one another on both maps. On both maps the supersuppressible mutants are distributed throughout the length of the gene. The other reverting mutants, however, are restricted to the right halves of the maps.

Additivity of the genetic fine structure maps: While the X-ray and meiotic data yield the same sequence of sites with few exceptions the maps differ with respect to the additivity of recombination frequencies. To facilitate the comparison of the maps the additivity of map segments consisting of widely separated and more closely linked mutants will be considered separately.

The crosses between the mutants shown in the maps of Figures 4 and 5 illus-

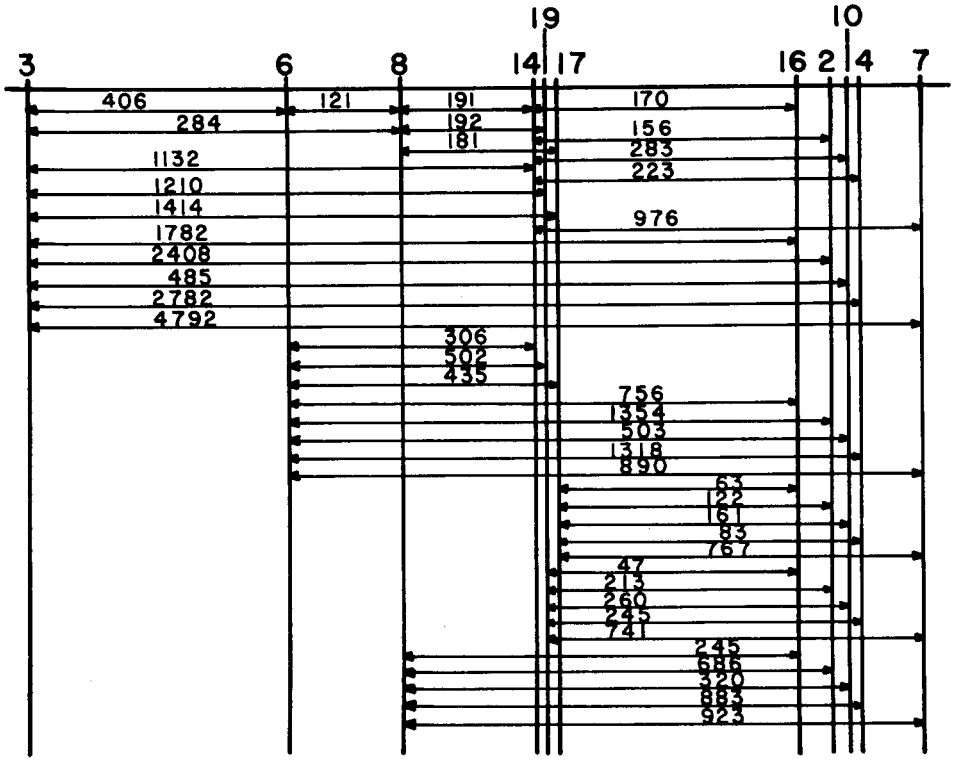

FIGURE 4.—Meiotic map of mutants *ad-8*–3, *8*–6, *8*–8, *8*–14, *8*–19, *8*–17, *8*–16, *8*–2, *8*–10, *8*–4 and *8*–7. The recombination frequencies are expressed as the number of prototrophs/10^5 asci.

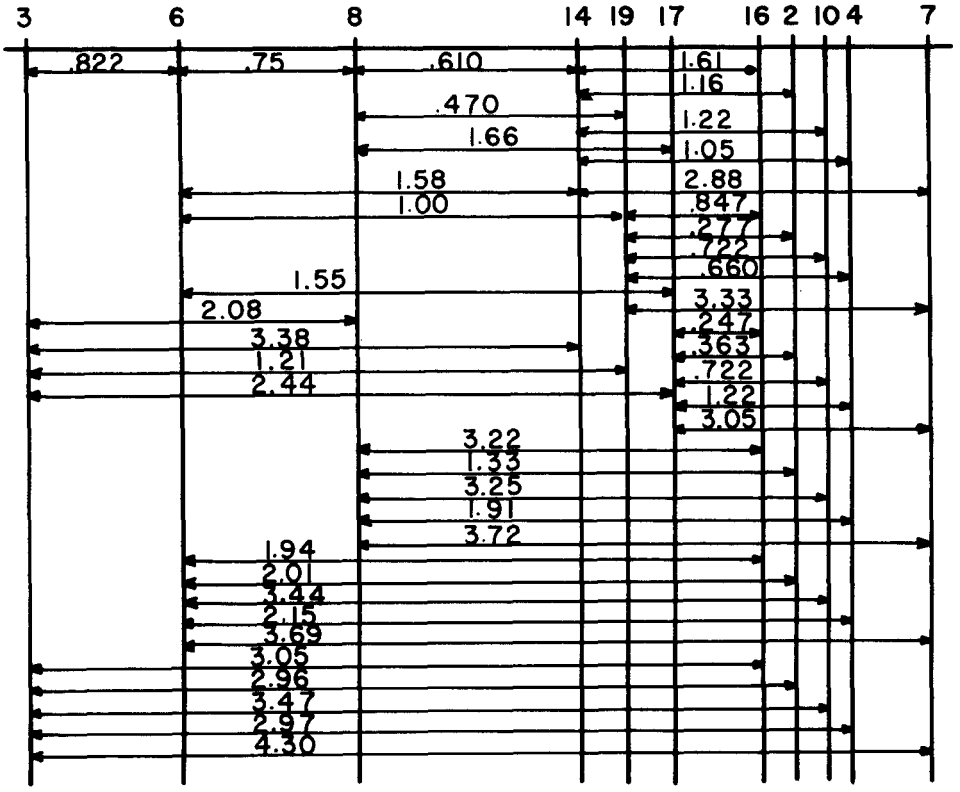

FIGURE 5.—X-ray map of mutants *ad-8–3*, *8–6*, *8–8*, *8–14*, *8–19*, *8–17*, *8–16*, *8–2*, *8–10*, *8–4* and *8–7*. The recombination rates are expressed as the number of prototrophs/10^8 cells/roentgen.

trate the properties of X-ray and meiotic mapping where the mutants are distant from one another.

The sequence of sites on both maps is the same. In each case the order shown is the most probable one. The mutants were considered three at a time and assigned an order on the basis of the three crosses between them. There were seventy combinations (henceforth referred to as triplets) to be considered. The crosses between the mutants which are not shown, involve very short intervals and do not enter into this calculation. The order of thirteen percent of the X-ray triplets and nineteen percent of the meiotic triplets contradict the sequence shown. Thus, in so far as the number of internal contradictions in the ordering of widely separated mutants is concerned, the X-ray and meiotic data are quite similar.

Inspection of the meiotic map of Figure 4 reveals that the estimate of the distance between two mutants as measured by their frequency of recombination usually exceeds the value that would have been predicted by summing the adjacent intervals between them. This tendency has been observed in other fungal fine structure maps and has been called "map expansion" by HOLLIDAY (1964).

To illustrate this tendency of the meiotic data the mutants were taken three at a time and the recombination frequency of the outermost pair of mutants (*a-c*) was plotted against the value expected by summing the two intervals *a-b* and *b-c*.

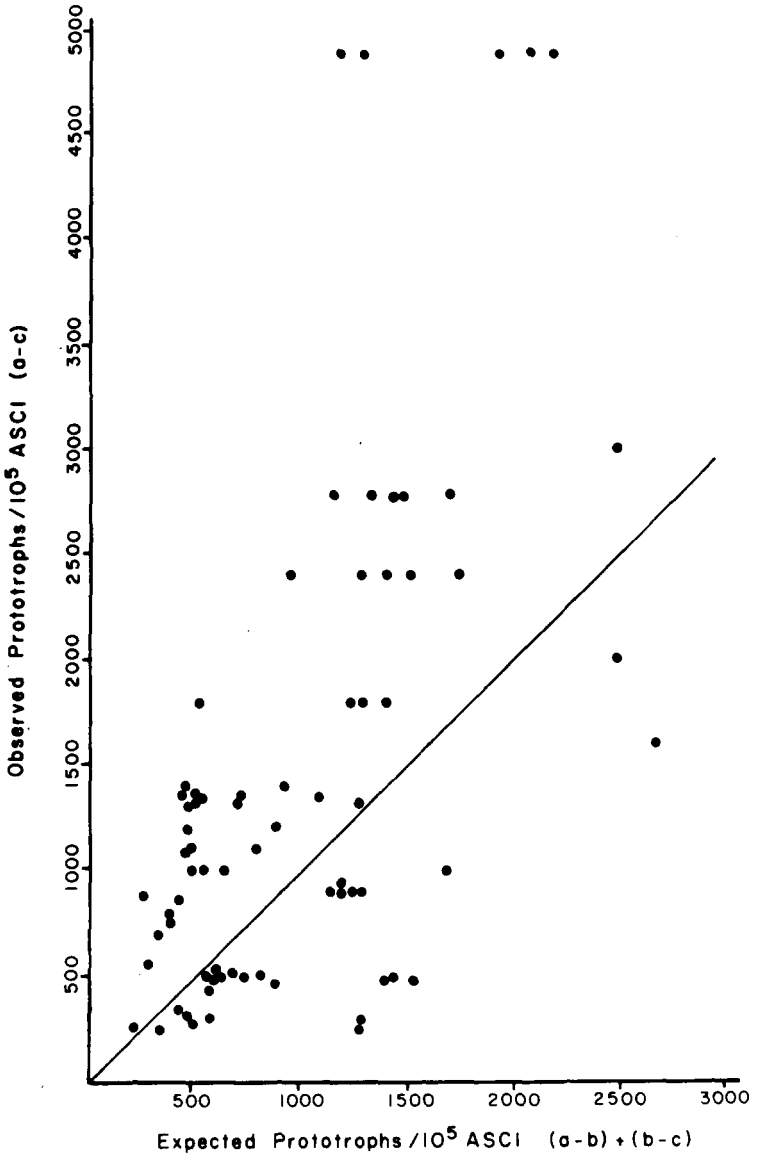

FIGURE 6.—Plot of observed and expected meiotic recombination frequencies for the mutants shown in the map of Figure 4. The line of slope 1 is drawn for reference.

This plot is shown in Figure 6. The observed values are plotted on the ordinate and the expected values on the abcissa. A line of slope 1 has been drawn for reference only to illustrate the departure of the data from additivity in the direction of map expansion.

The X-ray data were examined in the identical manner. The plot of observed reversion rates against the expected values for the X-ray crosses is shown in

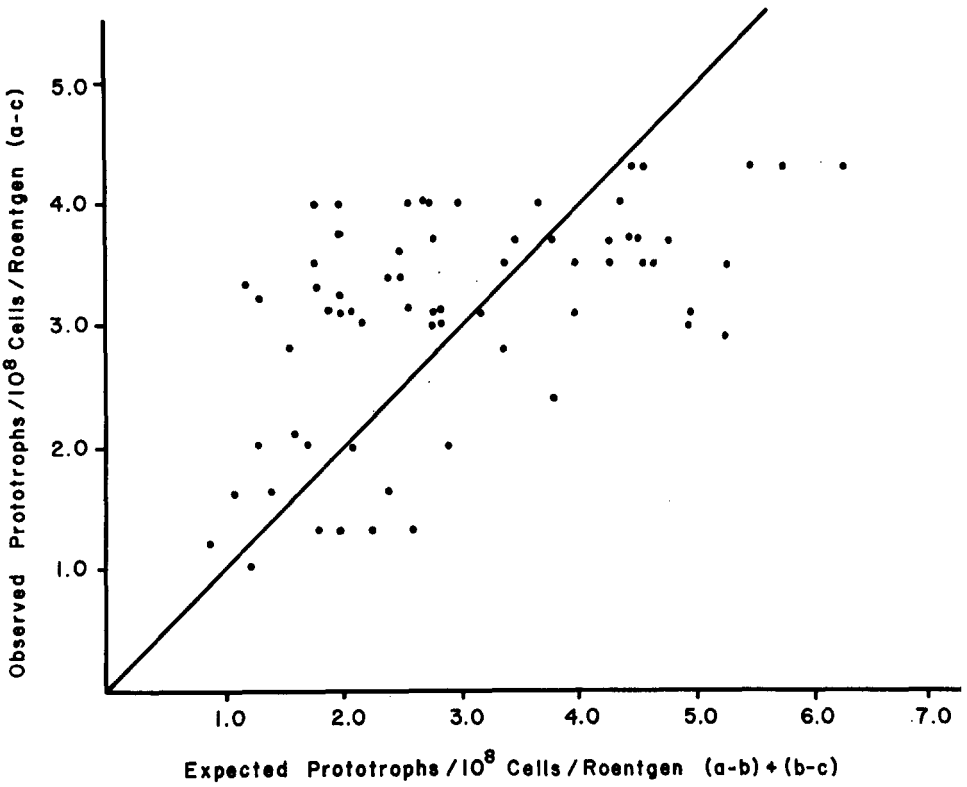

FIGURE 7.—Plot of observed and expected X-ray induced recombination rates for the mutants shown in the map of Figure 5. The line of slope 1 is drawn for reference.

Figure 7. The X-ray data show map expansion for the expected values up to about 3.0 prototrophs/10^8/r. Beyond this value the values fall below the line of slope 1. The map of Figure 5 reveals the source of this heterogeneity. Where three alleles are close to one another the recombination frequencies of the outermost pair tends to exceed the sum of the adjacent intervals. However, where the mutants are far apart, the sum of the two adjacent intervals usually exceeds the value observed in the cross of the outer pair of mutants. This latter trend will be referred to as "map contraction."

The meiotic and X-ray map of two short segments involving closely linked mutants, are shown in Figures 8 and 9. There are no internal contradictions in either meiotic map. In the X-ray maps, however, five of ten triplets contradict the order of the mutants of segment *a* and five of ten mutants contradict the order of mutants of segment *b*. Thus, the ordering of closely linked mutants by the X-ray method of mapping is less certain.

The expected and observed values of both meiotic maps of Figure 8 have been plotted in Figure 10. As in the mapping of more distantly separated mutants most of the points are above the line of slope 1, indicating a tendency toward map expansion. Most of the points, however, are closer to the line of slope 1 than

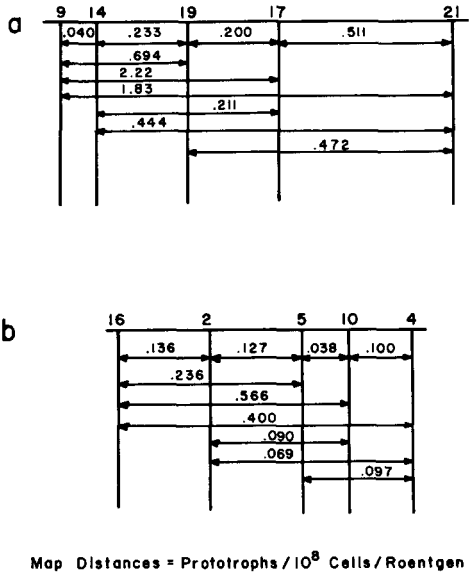

FIGURE 8.—Meiotic maps of two segments involving closely linked mutants. Map distances are given as the number of prototrophs/10^5 asci.

those in the plot of crosses involving mutants far apart, indicating that meiotic recombination frequencies of closely linked mutants are more additive.

The X-ray values for both short segments have been ploted together in Figure 11. The X-ray triplets involving closely linked intervals show a considerable

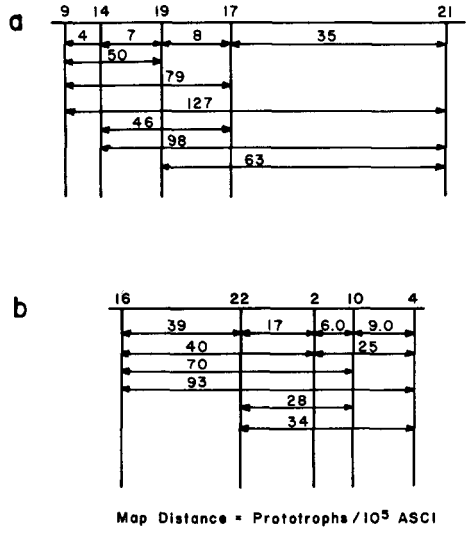

FIGURE 9.—X-ray maps of two segments involving closely linked mutants. Map distances are given as the number of prototrophs/10^8 cells/roentgen.

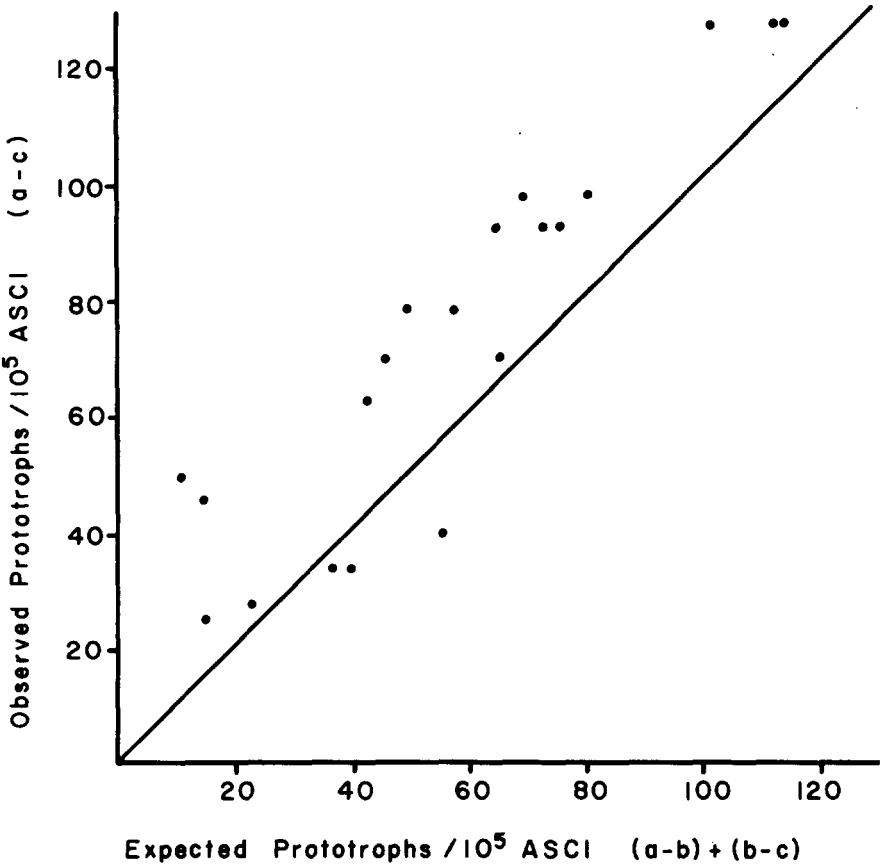

FIGURE 10.—Plot of observed and expected meiotic recombination values of crosses involving the mutants shown in Figure 8. The line of slope 1 is drawn for reference.

scattering of points with equal numbers of values above and below the line of slope 1. Here one cannot distinguish a trend toward either map contraction or map expansion.

The additivity of individual triplets: These trends in the meiotic and X-ray recombination data can be demonstrated without deciding upon a most probable overall order of sites, indicating that the departures from additivity are not an artifact of the attempt to construct a fine structure map.

The approach is to consider all possible combinations of three mutants which recombine and to assign to each triplet the order indicated by the recombination frequencies of the three crosses between them. Having ordered the mutants in the sequence *a-b-c*, the sum of the recombination frequencies of the crosses *a/b* and *b/c* is then divided by the recombination frequency of the outer pair of mutants, *a* and *c*. This quotient, $\frac{a/b + b/c}{a/c}$, is a measure of the additivity of the recombination frequencies of the crosses involved in the triplet, and will be referred to as the "coefficient of additivity."

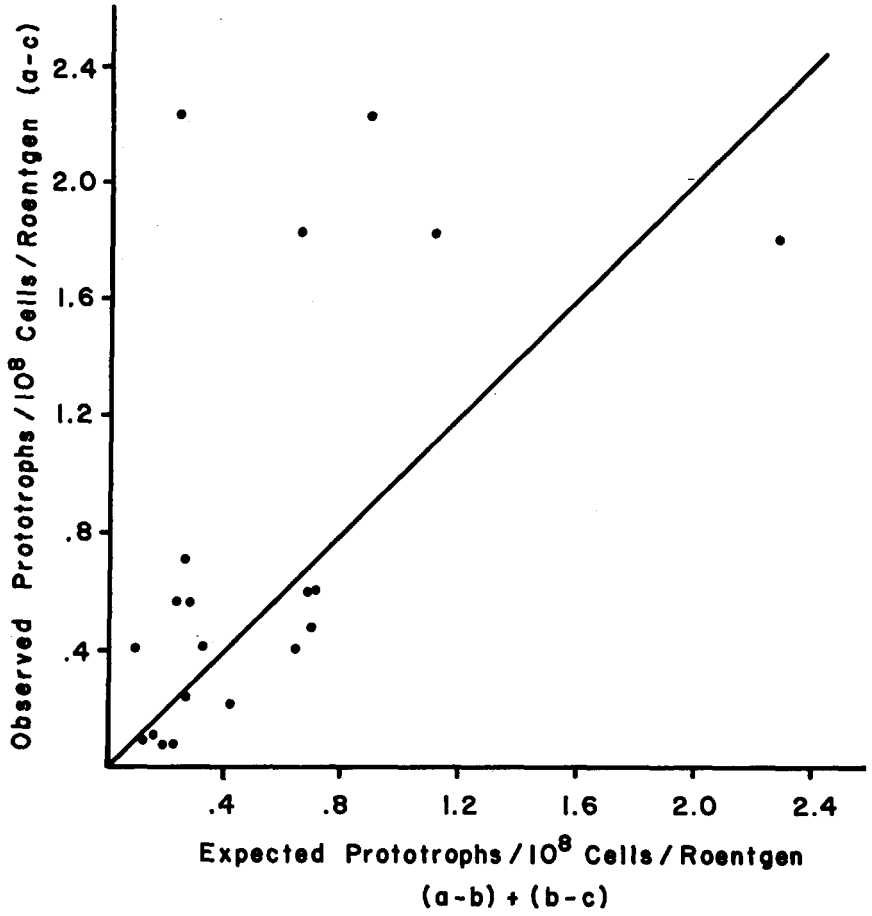

FIGURE 11.—Plot of observed and expected X-ray induced recombination values of crosses involving the mutants shown in Figure 9. The line of slope 1 is drawn for reference.

If the recombination frequencies of a set of two point crosses were additive one would expect that the distribution of the coefficients of additivity of the corresponding triplets would be distributed about a modal value of 1.0. On the other hand, if most of the triplets showed map expansion the mode would be at some value less than 1.0. Similarly, if the trend were primarily in the direction of map contraction the mode would be at some value greater than 1.0.

The coefficients of additivity of all of the triplets were calculated for both the meiotic and X-ray data using the values shown in Figures 1 and 2.

The array of meiotic coefficients of additivity has been plotted as a frequency distribution in Figure 12. 72% (943/1309) of the coefficients are less than 1.0, indicating a tendency toward map expansion in the meiotic data.

The frequency distribution of the coefficients of additivity of the X-ray triplets is shown in Figure 13. Here 58% (594/1033) of the coefficients are less than 1.0, and 32% (334/1033) are greater than 1.0. These results are in agreement with

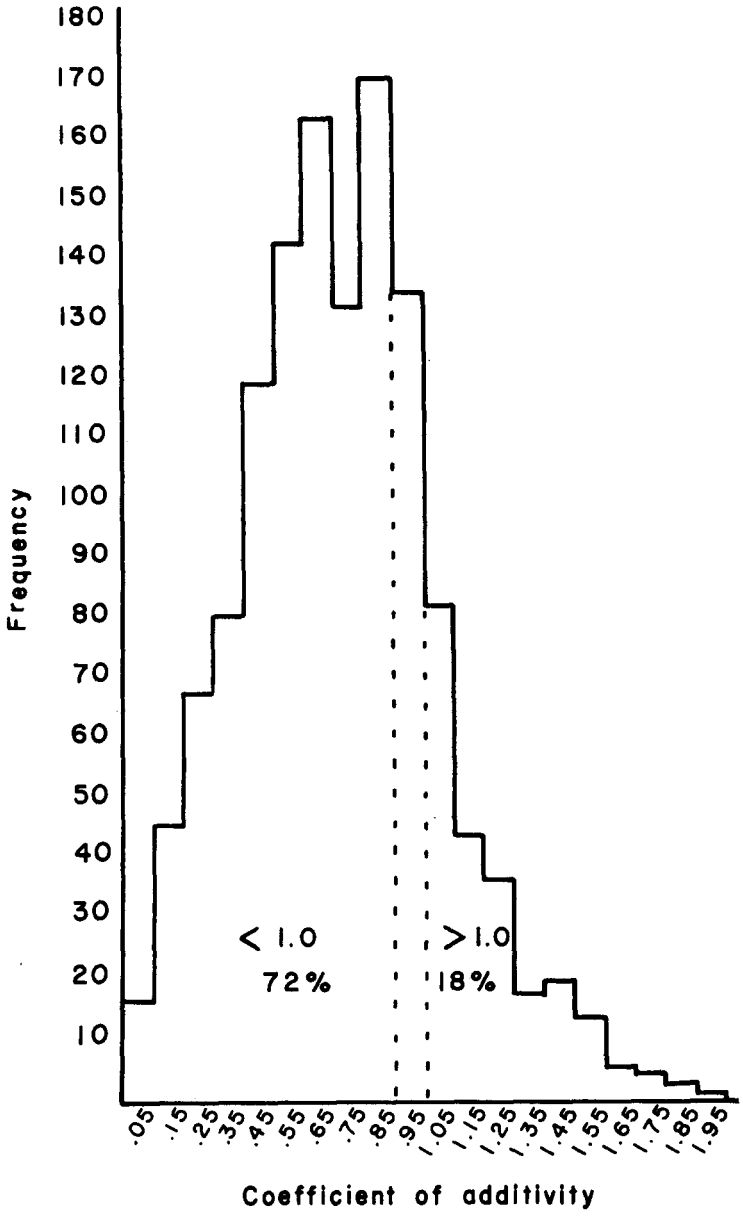

FIGURE 12.—Frequency distribution of the coefficients of additivity of meiotic triplets.

the properties of X-ray and meiotic recombination inferred from the maps.

The size of the ad-8 *gene:* As stated in the introduction the chief aim of this investigation was to obtain an estimate of the size of the adenine-8 gene to determine whether there was any correlation between its size and the low frequency with which it occurs among mutants affecting adenine biosynthesis.

1. *Recombinational length:* The genetic fine structure maps provide an esti-

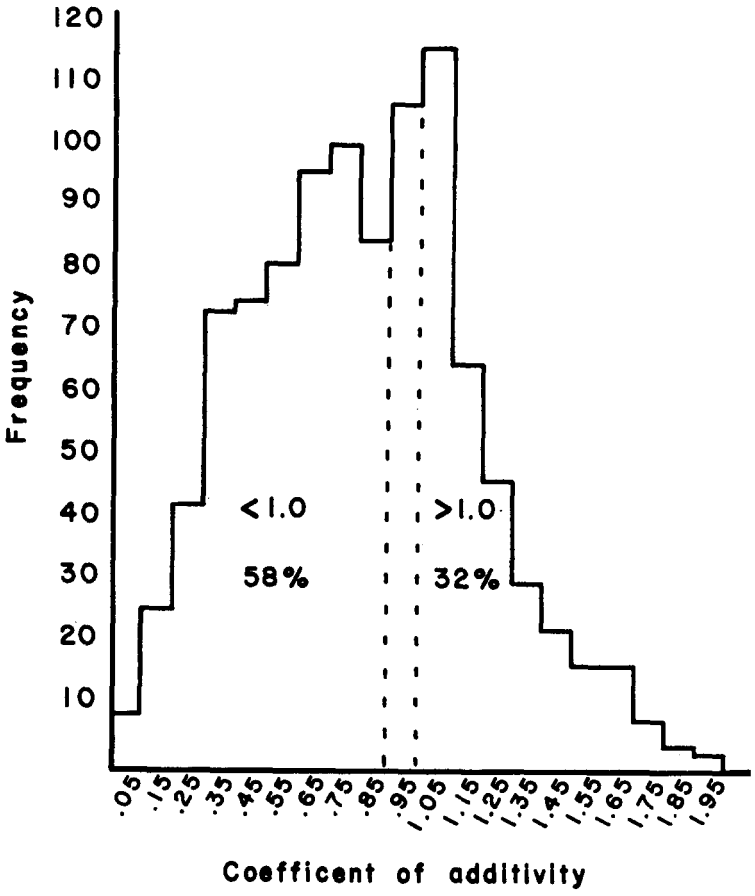

FIGURE 13.—Frequency distribution of the coefficients of additivity of X-ray triplets.

mate of this gene in terms of its recombinational length. The sum of the adjacent intervals of the meiotic map is 1033 prototrophs/10^5 asci. This value, however, is most likely an underestimate of its true length due to map expansion, since the recombination frequency of the terminal markers (*ad-8–3*/*ad-8–7*) is 4792 prototrophs/10^5 asci.

The sum of the adjacent intervals of the X-ray map is 4.64 prototrophs/10^8/r. This value as well is probably an underestimate due to map expansion; however, the recombination frequency of *ad-8–3* and *ad-8–7* is in greater agreement (4.30 prototrophs/10^8/r) due to the occurrence of map contraction over long intervals.

2. *Number of mutable sites:* An additional estimate of the size of the *ad-8* gene may be obtained by calculating the number of mutable sites from the frequency of crosses in which reverting mutants were not separable by recombination. The equation for the calculation of the number of mutable sites (n) is $n = N/I$, where N is the number of heteroallelic crosses tested, and I is the number of crosses in which mutants were not separated by recombination (DOBZHAN-

SKY and WRIGHT 1941; LEWONTIN and PROUT 1956). In the experiments reported here there were 6/190 crosses involving revertable mutants which failed to recombine following X irradiation and meiosis. The number of mutable sites by the formula given above is 32 ± 74.

3. *Number of nucleotide pairs:* MANNEY and MORTIMER (1963) have calculated that 1 X-ray unit (1 prototroph/10^8/r) is equivalent to a region of DNA of 150 nucleotide pairs coding for 50 amino acids. Based on their estimate, the *ad-8* gene contains approximately $(4.64 \times 150) = 696$ nucleotide pairs and codes a peptide of approximately 232 amino acids. Since no intragenic complementation has been observed at the *ad-8* locus in this study and in a previous investigation (DORFMAN 1964) it is likely that the locus codes a monomeric enzyme. The above calculations suggest a molecular weight of approximately 23,200 for the *ad-8* enzyme.

DISCUSSION

In this study the genetics of the *adenine-8* locus was investigated with a view to obtaining an understanding of why spontaneous mutations of this gene are infrequent. In a previous investigation JONES (1964) found a positive correlation between the gene size and mutation frequencies of two adenine loci, *ad-3* and *ad-6*. She found that the *ad-6* gene was approximately twice the size of the *ad-3* locus in terms of both recombinational length and number of mutable sites. This twofold difference, however, was not sufficient to explain why mutants of the *ad-6* locus are five to ten times more frequent than those of *ad-3*. The observation that nearly all mutants of *ad-3* had retained partial activity suggested that this difference might be due to the failure to recover *ad-3* mutants.

The *ad-8* locus was chosen for study to compare its size with that of *ad-3* and *ad-6*. Mutants of *ad-8* occur with approximately the same frequency as those of *ad-3*; however, like *ad-6*, mutants of *ad-8* with apparently complete loss of function are recovered. It was therefore thought that the recoverability of mutants of *ad-8* would be the same as that of *ad-6*. If so one might expect a closer correlation between *ad-8* and *ad-6* in relation to mutability versus gene size if the latter is an important component of mutation frequency.

The ratios of the spontaneous mutation frequencies, meiotic map lengths, and numbers of mutable sites of *ad-8*, *ad-6*, and *ad-3* are summarized in Table 4. While the properties of the *ad-8* mutants suggested that one would observe a closer correlation between the gene size of *ad-8* and *ad-6* and their spontaneous mutation frequencies, it can be seen that even in the case of these loci the differences in map length are not sufficient to explain the differential mutability of these loci. *ad-8* was found to be approximately one third the length of *ad-6* but *ad-8* yields one sixth as many mutants.

A comparison of the distribution of the various classes of mutants on the maps of *ad-8* and *ad-6* suggests a possible interpretation of this discrepancy. In this study the putative missense mutants of *ad-8* (reverting, nonsupersuppressible) occupy primarily the right half of the gene map. On the fine structure map of *ad-6* the mutants of this class are distributed throughout the length of the locus.

TABLE 4

Comparison of spontaneous mutation frequencies, meiotic map lengths and numbers of mutable sites of adenine-3, adenine-6 and adenine-8

	No. of mutants mapped	Map length^{+}/10^{5} asci	Number of mutable sites	Reference
adenine-3	25	1690	101 ± 30	Jones (1964)
adenine-6	26	3239	195 ± 33	Jones (1964)
adenine-8	22	1033	32 ± 74	Esposito (1968)
	ad-3/ad-8	*ad-6/ad-3*	*ad-6/ad-8*	
Ratio of meiotic map lengths	$\frac{1690}{1033} = 1.6$	$\frac{3239}{1690} = 1.9$	$\frac{3239}{1033} = 3.2$	.....
Ratio of mutable sites	$\frac{101}{32} = 3.2$	$\frac{195}{101} = 1.9$	$\frac{195}{32} = 6.1$	.....
Ratio of numbers of spontaneous mutants recovered	$\frac{106}{98} = 1.1$	$\frac{592}{106} = 5.6$	$\frac{592}{98} = 6.0$	Esposito (1968)
Ratio of numbers of spontaneous mutants recovered	$\frac{67}{99} = .7$	$\frac{571}{67} = 8.5$	$\frac{571}{99} = 5.8$	Jones (1964)

Thus, in the case of *ad-8*, the spontaneous mutation frequency of this gene may not be a true reflection of its size because a high proportion of the missense mutations occurring in the left half of the gene do not result in a mutant phenotype.

The ratio of the numbers of mutable sites of *ad-6* and *ad-8* is in closer agreement with the ratio of their mutation frequencies. This result, however, is probably not significant due to the large error involved in the calculation of the mutable site estimates.

The fact that *ad-8* is smaller than *ad-3* both in terms of number of mutable sites and recombinational length, while it produces a similar number of mutants, is consistent with the interpretation that a large fraction of *ad-3* mutations are not recovered.

The genetic fine structure maps: While the results of mapping using X-ray induced mitotic allelic recombination data and meiotic allelic recombination frequencies generated maps with essentially the same order of sites, the properties of the maps were found to differ with respect to the additivity of allelic recombination frequencies. The meiotic recombination frequencies of closely linked mutants were more additive than those involving distantly separated mutants. In crosses of mutants far apart the recombination frequency usually exceeded the value expected by summing adjacent intervals to a far greater extent than was observed in the case of more closely linked alleles.

The tendency for the recombination frequency of a pair of alleles to exceed the value predicted by summing adjacent intervals first received attention from STADLER (1960) who illustrated this trend in the fine structure map of allelic cysteine requiring mutants of Neurospora. This phenomenon has been called map expansion by HOLLIDAY (1964) who indicated its widespread occurrence in

the fine structure maps of several fungi. HOLLIDAY (1964) has suggested that map expansion represents the inhibitory effect of a mutant allele on recombination in its vicinity and more recently that it is the consequence of the excision of regions of hybrid DNA during the recombination process (1966).

In the original description of the technique of X-ray mapping (MANNEY and MORTIMER 1963) these authors concluded that X-ray induced allelic recombination data were consistently additive. The results of the present study indicate that this is not the case for the *ad-8* locus. The X-ray induced recombination rates of closely linked mutants showed several departures from additivity. Crosses between more distantly separated sites could be divided into two classes. Those in which the expected recombination value was less than 3.0 prototrophs/10^8/r showed a trend toward map expansion; those in which the expected value was greater than 3.0 prototrophs/10^8/r usually gave fewer recombinants than expected. While this latter tendency may be interpreted to be the result of multiple recombinational events over long intervals, it has been referred to by the neutral term, map contraction, in the absence of a test of this hypothesis.

Map contraction in the X-ray crosses of widely separated mutants resembles what is observed in genetic fine structure maps of bacteria and phage (STREISINGER and FRANKLIN 1956; BENZER 1957; CHASE and DOERMAN 1958; YANOFSKY 1960; GAREN 1960; EDGAR *et al.* 1962; AMATI and MESELSON 1965; FISHER and BERNSTEIN 1965). Likewise the difficulty in ordering closely linked mutants on the X-ray maps recalls the non-linearity of recombination frequencies observed by TESSMAN (1965) in attempting to order closely linked sites of the *rII* locus of phage T4. These similarities raise the possibility that the mechanism of recombination in mitotic cells of yeast is intermediate between that observed in meiosis and that observed in organisms with less complex chromosomes.

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SUMMARY

To investigate the relationship between the size of the *ad-8* gene and its low spontaneous mutability, the size of the locus was estimated by two independent criteria of gene size: recombinational length and number of mutable sites. Two methods of mapping were employed to determine the recombinational length of the locus. A meiotic fine structure map was constructed using allelic recombination frequencies after meiosis as a measure of the distance between mutant sites and an X-ray map was constructed from X-ray induced allelic recombination rates of mitotically grown diploids. The length of the meiotic fine structure map was $1033^+/10^5$ asci and the length of the X-ray map was $4.64^+/10^8$ cells/roentgen. The number of mutable sites at the *ad-8* locus, based upon the frequency of crosses involving reverting mutants which failed to recombine, was 32 ± 74. The

size of the *ad-8* gene in terms of meiotic map length and number of mutable sites was compared to that of *ad-6* which yields five to ten times as many spontaneous mutants as *ad-8*. The results of this comparison indicated that the low spontaneous mutability of *ad-8* could be explained only in part by the difference in the size of these loci. The distribution of putative missense mutations on the *ad-8* fine structure map indicated that this discrepancy might be due to the failure to recover appreciable numbers of missense mutants from one half of the *ad-8* gene. —While both the X-ray and meiotic mapping of *ad-8* mutants generated the same sequence and distribution of sites with few exceptions, they differed in resolution and additivity. Meiotic allelic recombination frequencies involving closely linked mutants were more additive than those involving markers further apart. Both crosses, between markers close together and those more distant from one another, demonstrated a tendency toward "map expansion," i.e., the recombination frequency of a pair of mutants usually exceeded the value expected by summing the adjacent intervals between them. In the X-ray mapping, however, crosses involving closely linked mutants demonstrated a greater departure from additivity than those involving more distantly separated sites. The latter crosses could be divided into two classes. Those in which the expected value was less than $3.0^{+}/10^{8}$ cells/roentgen showed a tendency toward "map expansion." Those in which the expected value was greater than $3.0^{+}/10^{8}$ cells/roentgen showed a tendency toward "map contraction," i.e., the value observed was less than the value expected by summing adjacent intervals.

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