INTERALLELIC RECOMBINATION AT THE *his-5* LOCUS IN *NEUROSPORA CRASSA*

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1. INTRODUCTION

THE *histidine-5* gene of *Neurospora crassa* is situated in the right arm of chromosome IV between *pyrimidine-3* and *tryptophan-4* (Smith, 1962). The gene apparently controls the primary structure of the enzyme glutamate-phosphohistidinol transaminase which catalyses the conversion of imidazole acetol phosphate to L-histidinol phosphate (Ames and Horecker, 1956).

A considerable number of *his-5* auxotrophic mutants are known some of which exhibit complementation. The complementation matrix of 60 *his-5* mutants was interpreted in a linear complementation map within which eight distinct complementing groups were apparent (Catcheside, 1960). Study of the complementation characteristics of these 60 *his-5* alleles and a further 30 has revealed only slight changes in this complementation map (p. 271). The complementation map is not so simple, nor the number of alleles so few that an exact colinearity between genetic and complementation maps might be expected by chance, and to test this possibility was the main reason for attempting to map the fine structure of *his-5*.

Mapping of the sites of difference in the gene depends primarily on the frequencies of prototrophs found amongst ascospores produced by crosses between pairs of auxotrophs in the repulsion phase. This assumes that the frequency of prototrophs found in such crosses is a measure of the physical distance between the sites at which the two auxotrophs differ from one another. Thus any pair of auxotrophic alleles, m^1 and m^2 , would show a frequency which was the sum or the difference of the frequencies shown by crosses of these alleles with a third auxotroph. If this additivity rule held, it should be possible to arrange the sites of mutation in an unambiguous order.

In this work, mapping was not solely dependent upon prototroph frequency, but also on the distribution of the combinations of flanking markers amongst the prototrophs produced by crosses. In a cross of the general type $Am^1B \times am^2b$ where A and a are the proximal and B and b are the distal flanking markers, the prototrophs, m^+ fall into four classes. These are the two parental combinations AB and ab and

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the two recombinants Ab and aB . In *Neurospora* no one recombinant class vastly exceeds any other class and all four occur with substantial frequencies. However, some asymmetries are observed. The recombinant classes are often unequal in size. The parental classes are often unequal in size, and the directions of inequality of the parental and recombinant classes are correlated. It has often been assumed that the inequalities are due to "normal" crossing-over and that the high frequencies of parental and the second recombinant classes are due to the simultaneous occurrence of two or three crossovers indicating a high degree of "negative interference". However, high negative interference has been invoked merely as a description of the factor which is necessary to fit the observations to the crossover theory

FIG. 1.—Diagram to show the derivation of the criteria for ordering the sites of difference m^1 and m^2 based on the distribution of flanking markers A and B amongst prototrophic recombinants m^+ .

of recombination. The main concern of this paper is not so much with the theory of recombination as with the ways in which the data may be used to order the sites of mutation.

The relationships used to indicate the order of the sites of mutation are best understood if it is assumed that recombination does occur by a process analogous to crossing-over. Three criteria may be considered.

1. If the recombinant classes are compared, the order will be Am^1m^2B if aB is in the majority and will be Am^2m^1B if Ab is in the majority (see fig. 1).

2. If the proximal flanking alleles only are considered, the order will be Am^1m^2 if a is in the majority and will be Am^2m^1 if A is in the majority. In other words, the more frequent proximal flanking allele identifies the more distal of the m allele differences since they entered the cross together.

3. If the distal flanking alleles are considered, the order will be m^1m^2B if B is in the majority and will be m^2m^1B if b is in the majority. That is, the more frequent distal flanking allele identifies the more proximal of the m allele differences since they entered the cross together.

The first criterion uses only the recombinant classes, whereas the second and third criteria also use the parental classes. Hence although the second and third criteria are not wholly independent of the first, they do have the merit of using all the progeny scored.

If any, or all, of these criteria lead to the derivation of a consistent order for a series of alleles, this order must be considered as meaningful. Consistency may be expected to hold between a series of pairs of a set of alleles considered with respect to one criterion and between the three criteria applied to one pair of alleles. Inconsistency can only be considered significant if the order inferred is based on a significant difference in frequency between the classes of prototrophs compared.

2. MATERIALS AND METHODS

(a) *Organisms*

The *his-5* mutants comprise 90 alleles, 88 of which were isolated by Catcheside from *Emerson a* by filtration enrichment following irradiation with ultra-violet light.

TABLE 1

Histidine-5 alleles and their complementation groups

Complementation group	Number of alleles in each group	Mutant isolation number
A	44	K50, K171, K265, K268, K269, K514, K517, K519, K523, K530, K531, K532, K534, K537, K539, K540, K548, K550, K551, K552, K553, K585, K963, K997, K1000, K1004, K1074, K1085, K1132, K1149, K1186, K1187, K1195, K1199, K1214, K1232, K1233, K1251, K1267, K1283, K1339, K1361, K1397, K1559
B	2	K516, K746
C	1	K877
D	10	K52, K54, K271, K447, K527, K535, K536, K1249, K1266, K1390
E	7	K522, K529, K538, K543, K736, K873, K1500
F	4	K541, K1412, K1422, K1552
G	17	K71, K78, K155, K201, K255, K524, K542, K726, K728, K729, K730, K731, K732, K733, K734, K735, K1378
H	1	K512
I	4	K513, K521, K533, K546
	Total 90	

The remaining two, K873 and K877, were isolated by the author after irradiation of the same wild type with gamma rays. The *his-5* mutants are listed in table 1. The *pyrimidine-3* mutant used was 1298 originally received from Dr G. W. Beadle. The *leucine-2* mutant 37501 was kindly donated by Dr D. D. Perkins. *Pyr-3* is about one map unit proximal to *his-5* and *leu-2* about nine map units distal to *his-5*.

(b) *Media*

All stocks were grown and kept on slopes of Vogel's medium (Vogel, 1955), suitably supplemented. Crosses were made on slopes of Westergaard's medium (Westergaard and Mitchell, 1947) containing folded strips of filter paper. Cultures were tested for biochemical requirements on Vogel's medium in which 0.5 per cent. sorbose and 0.1 per cent. sucrose replaced the usual 2 per cent. sucrose. Supplements of growth factors were added to these media where necessary, to give the

following concentrations; for histidine mutants, 300 mg./l. of L-histidine monohydrochloride, for the *pyr-3* mutant 500 mg./l. of uracil and for the *leu-2* mutant 600 mg./l. of DL-leucine.

(c) Crosses

Each cross for analysis of prototroph frequency was made in seven to twenty tubes of crossing medium and ascospores from the different tubes were pooled. The ascospores were collected in sterile water, filtered through muslin, and after removal of excess water by decantation suspended in 0.1 per cent. agar. The number of spores per ml. of suspension was estimated by counting the number in 0.01 ml. drops of suspension on slides on which the area covered by the coverslip was divided into sixty-four squares. The ascospores were screened for prototrophs by a modification of the layer plating technique (Newmeyer, 1954; Murray, 1960). Germination of the ascospores suspended in 0.1 per cent. agar was induced by heat treatment at 56° C. for 60 minutes. Known volumes of spore suspension were then pipetted into tubes containing 2.5 ml. volumes of 0.5 per cent. agar supplemented with uracil and leucine which was kept molten at 45° C. The contents of each tube were quickly mixed and evenly spread on plates of Vogel's medium supplemented with uracil and leucine. The plates were then incubated at 25° C. and scanned under a stereomicroscope after 18 hours, 42 hours and 66 hours. Prototrophs were transferred to slopes of Vogel's medium containing uracil and leucine and later classified for *pyr-3* and *leu-2*. The viability of the ascospores of each cross was estimated in samples layer plated on Vogel's medium plus histidine, uracil and leucine.

3. RESULTS

Crosses of the type *pyr-3 his-5*^{*} + X + *his-5*^y *leu-2* were prepared, where *his-5*^{*} and *his-5*^y designate two *his-5* alleles and *pyr-3* (1298) and *leu-2* (37501) are proximal and distal markers respectively. Spores from such crosses were analysed for their frequency of prototroph formation. The prototrophs were then classified for the four combinations of flanking markers, the two parental *pyr-3* + and + *leu-2*, and the two recombinant, ++ and *pyr-3 leu-2*. The data are summarised in table 2. The important statistics in this table are (i) the frequencies of prototrophs per hundred thousand ascospores analysed in a cross and (ii) the order of the *his-5* allele differences as deduced from the distribution of the four classes of flanking marker combinations amongst these prototrophs. These two aspects of the data are considered separately.

(a) Prototroph frequencies

The observed frequencies of prototrophs ranged from zero to 35 or so per 10⁵ ascospores. Of eight self crosses, crosses 1 to 8 in table 2, only one yielded any prototrophs. This was the self cross K550 × K550* which yielded one prototroph in 18.6 × 10⁵ ascospores. This was taken to indicate a higher reversion rate of K550 than is usual for most *his-5* mutants. We shall see (p. 226) that this is so.

The data include twelve sets of two or more crosses between

* In writing the constitution, only the allele numbers of the *his-5* mutants will be given. Strictly the constitution here should be *his-5*K550.

particular pairs of alleles where the stocks were reciprocally or independently marked. These sets of families are bracketed in table 2. In three of these sets, the frequency of prototroph formation in reciprocally marked crosses did not differ significantly. These were

TABLE 2

Cross		<i>his-5</i> prototrophs				Proximal allele difference by criterion			
Serial no.	Constitution <i>pyr-3</i> × <i>leu-2</i>	Number per 10 ⁵ viable ascospores	Distribution of flanking markers				I	II	III
			<i>pyr</i>	<i>leu</i>	++	<i>pyr leu</i>			
1	K550* × K550 7	0.054	0	0	1	0			
2	K746 1 × K746 1	(4444)***	0	0	0	0			
3	K516 1 × K516 1	(3748)	0	0	0	0			
4	K268 5 × K268 1	(211)	0	0	0	0			
5	K553 1 × K553 1	(792)	0	0	0	0			
6	K729 2 × K729 1	(4867)	0	0	0	0			
7	K540 1 × K540 1	(2998)	0	0	0	0			
8	K512 1 × K512 1	(4476)	0	0	0	0			
9	K746 1 × K550 11	11.6 ± 2.0	4	16	15	0	K746	K746	K746
10	K268 5 × K550 2	12.6 ± 1.7	17	27	11	0	K268	K268	K268
11	K78 1 × K550 8	18.5 ± 1.8	12	51	39	5	K78	K78	K550
12	K553 1 × K550 7	8.7 ± 0.92	9	47	28	6	K553	K553	K550
13	K542 1 × K550 8	0.114	0	5	3	0	K542	K542	K550
14	K729 3 × K550 1	0.091	1	3	0	0	—	K729	K550
15	K540 1 × K550 7	19.2 ± 1.9	12	58	16	13	K540	K540	K550
16	K533** × K550 3	13.8 ± 1.6	0	78	0	0			
17	K512 1 × K550 8	10.7 ± 1.9	7	9	1	13	K550	K550	K550
18	K548 1 × K550 2	35.7 ± 2.8	62	47	20	35	K550	K550	=
19	K746 1 × K516 1	0.123 ± 0.034	0	3	10	0	K746	K746	K746
20	K268 1 × K746 1	1.5 ± 0.42	1	11	0	1	K746	K268	K746
21	K746 1 × K553 3	4.3 ± 0.6	9	12	30	1	K746	K746	K746
22	K553 1 × K746 1	6.47 ± 0.57	17	27	11	71	K746	K746	K746
23	K746 1 × K450 1	8.8 ± 0.89	15	22	61	1	K746	K746	K746
24	K746 1 × K548 3	18.0 ± 1.7	9	46	59	1	K746	K746	K746
25	K268 5 × K516 3	1.38 ± 0.35	7	1	0	8	K516	K516	K516
26	K553 1 × K516 1	6.4 ± 0.59	17	26	9	65	K516	K516	K516
27	K553 1 × K516 2	4.8 ± 0.43	21	22	4	74	K516	K516	K516
28	K516 1 × K553 3	7.1 ± 0.74	8	10	69	4	K516	K516	K516
29	K540 1 × K516 1	9.3 ± 1.3	7	8	3	33	K516	K516	K516
30	K540 1 × K516 2	13.3 ± 0.8	76	55	21	122	K516	K516	K516
31	K516 1 × K540 1	12.9 ± 1.3	36	47	99	12	K516	K516	K516
32	K548 1 × K516 3	37.6 ± 1.9	145	24	19	219	K516	K516	K516
33	K516 1 × K548 3	35.7 ± 4.8	2	21	28	4	K516	K516	K516
34	K268 1 × K78 1	0.63	0	0	3	0	K268	K268	K268
35	K268 2 × K155 2	1.06 ± 0.34	0	4	6	0	K268	K268	K268
36	K553 2 × K268 1	1.12 ± 0.34	1	5	0	6	K268	K268	K268
37	K729 1 × K268 1	4.98 ± 0.66	17	11	3	25	K268	K268	K268
38	K268 5 × K540 3	5.03 ± 0.75	7	22	13	3	K268	K268	K540
39	K548 1 × K268 1	24.3 ± 2.1	34	23	5	71	K268	K268	K268

families 32 and 33, 40 and 41 and 63 and 64. In the other nine sets, frequencies of prototroph formation differed significantly between families. In no case was the highest frequency more than double the lowest frequency for crosses between the same pair of alleles. The

greatest difference was between families 29 and 31 for crosses between the reciprocally marked *his-5* alleles K₅₄₀ and K₅₁₆, which gave frequencies of 9.3 ± 1.3 and 17.9 ± 1.3 respectively. The difference, 8.6 ± 1.8 is highly significant ($P < 0.000,01$).

Within each of the 12 sets of families the relative distribution of the prototrophs amongst the four marker classes did not vary significantly. This indicates that the variations in frequencies do

TABLE 2—(continued)

Cross		<i>his-5</i> prototrophs					Proximal allele difference by criterion		
Serial no.	Constitution <i>pyr-3</i> × <i>leu-2</i>	Number per 10 ⁸ viable ascospores	Distribution of flanking markers				I	II	III
			<i>pyr</i>	<i>leu</i>	++	<i>pyr leu</i>			
40	{ K ₇₈ 1 × K ₁₅₅ 1	(4619)	0	0	0	0			
41	{ K ₁₅₅ 1 × K ₇₈ 1	(1815)	0	0	0	0			
42	{ K ₅₅₃ 1 × K ₇₈ 1	1.82 ± 0.29	9	7	6	17	K ₇₈	K ₇₈	K ₇₈
43	{ K ₇₈ 1 × K ₇₂₉ 1	5.01 ± 0.59	11	11	48	2	K ₇₈	K ₇₈	K ₇₈
44	{ K ₇₂₉ 2 × K ₇₈ 1	2.78 ± 0.22	27	27	8	92	K ₇₈	K ₇₈	K ₇₈
45	{ K ₇₈ 1 × K ₅₄₀ 2	8.35 ± 0.96	17	23	29	6	K ₇₈	K ₇₈	K ₇₈
46	{ K ₅₄₀ 1 × K ₇₈ 1	4.65 ± 0.75	8	7	1	23	K ₇₈	K ₇₈	K ₇₈
47	{ K ₇₈ 1 × K ₅₄₈ 1	30.5 ± 4.0	7	23	27	2	K ₇₈	K ₇₈	K ₇₈
48	{ K ₅₅₃ 1 × K ₁₅₅ 1	1.74 ± 0.44	3	4	1	8	K ₁₅₅	K ₁₅₅	K ₁₅₅
49	{ K ₅₄₂ 1 × K ₁₅₅ 1	2.1 ± 0.25	17	11	3	40	K ₁₅₅	K ₁₅₅	K ₁₅₅
50	{ K ₇₂₉ 2 × K ₁₅₅ 1	2.16 ± 0.27	16	7	2	40	K ₁₅₅	K ₁₅₅	K ₁₅₅
51	{ K ₅₄₀ 1 × K ₁₅₅ 1	3.33 ± 0.56	3	17	0	15	K ₁₅₅	K ₁₅₅	K ₁₅₅
52	{ K ₁₅₅ 1 × K ₅₄₈ 3	23.3 ± 4.76	2	6	16	0	K ₁₅₅	K ₁₅₅	K ₁₅₅
53	{ K ₅₅₃ 1 × K ₅₄₂ 1	1.54 ± 0.4	0	7	8	0	K ₅₅₃	K ₅₅₃	K ₅₅₃
54	{ K ₅₅₃ 1 × K ₇₂₉ 1	1.22 ± 0.29	1	5	12	0	K ₅₅₃	K ₅₅₃	K ₅₅₃
55	{ K ₅₅₃ 1 × K ₅₄₀ 1	3.8 ± 0.26	25	70	83	25	K ₅₅₃	K ₅₅₃	K ₅₅₃
56	{ K ₅₄₀ 1 × K ₅₅₃ 3	2.2 ± 0.18	38	22	15	63	K ₅₅₃	K ₅₅₃	K ₅₅₃
57	{ K ₅₄₀ 1 × K ₅₅₃ 4	2.6 ± 0.25	20	21	6	61	K ₅₅₃	K ₅₅₃	K ₅₅₃
58	{ K ₅₅₃ 1 × K ₅₃₃ 2	5.6 ± 0.82	5	17	15	9	K ₅₅₃	K ₅₃₃	K ₅₃₃
59	{ K ₅₅₃ 1 × K ₅₁₂ 1	3.85 ± 0.59	4	12	27	0	K ₅₅₃	K ₅₅₃	K ₅₅₃
60	{ K ₅₄₈ 1 × K ₅₅₃ 2	13.1 ± 1.0	74	13	5	65	K ₅₅₃	K ₅₅₃	K ₅₄₈
61	{ K ₅₅₃ 1 × K ₅₄₈ 1	16.2 ± 1.26	14	62	76	13	K ₅₅₃	K ₅₅₃	K ₅₅₃
62	{ K ₅₅₃ 1 × K ₅₄₈ 3	16.0 ± 1.2	19	59	83	17	K ₅₅₃	K ₅₅₃	K ₅₅₃
63	{ K ₅₄₂ 1 × K ₇₂₉ 1	(2771)	0	0	0	0	—	—	—
64	{ K ₇₂₉ 1 × K ₅₄₂ 2	0.028	2	0	0	0	—	K ₅₄₂	K ₇₂₉
65	{ K ₅₄₀ 1 × K ₅₄₂ 1	0.61	2	2	0	1	K ₅₄₂	K ₅₄₂	K ₅₄₂
66	{ K ₅₄₂ 1 × K ₅₄₈ 1	8.43 ± 1.8	4	8	9	1	K ₅₄₂	K ₅₄₂	K ₅₄₂
67	{ K ₅₄₀ 1 × K ₇₂₉ 1	0.49 ± 0.13	0	2	3	9	K ₇₂₉	K ₇₂₉	K ₇₂₉
68	{ K ₅₄₀ 1 × K ₅₅₃ 2	2.25 ± 0.49	3	7	7	4	K ₅₄₀	K ₅₄₀	K ₅₃₃
69	{ K ₅₄₀ 1 × K ₅₁₂ 1	3.5 ± 0.5	11	23	12	4	K ₅₄₀	K ₅₄₀	K ₅₁₂
70	{ K ₅₁₂ 3 × K ₅₄₀ 2	6.17 ± 0.64	(94 not tested)						
71	{ K ₅₄₀ 1 × K ₅₄₈ 1	14.9 ± 0.67	81	216	146	45	K ₅₄₀	K ₅₄₀	K ₅₄₈
72	{ K ₅₄₈ 1 × K ₅₄₀ 3	9.9 ± 1.1	35	18	7	22	K ₅₄₀	K ₅₄₀	K ₅₄₈
73	{ K ₅₁₂ 1 × K ₅₃₃ 2	0.031	0	0	0	2	K ₅₃₃	K ₅₃₃	K ₅₃₃
74	{ K ₅₄₈ 1 × K ₅₃₃ 3	2.74 ± 0.41	17	6	2	20	K ₅₃₃	K ₅₃₃	K ₅₃₃
75	{ K ₅₁₂ 1 × K ₅₄₈ 2	3.69 ± 0.85	4	5	7	3	K ₅₁₂	K ₅₁₂	K ₅₁₂

* This component did not contain any marker mutant.

** This component contained the *leu-2* marker mutant 37501.

*** In crosses where no prototrophs were found the number of viable ascospores screened in thousands is given in brackets. For each cross the total number of prototrophs obtained may be found by adding the four classes of prototrophs.

not result from differences in the viability of the four marker combinations. It seems probable that the differences between these families has a genetic basis.

When considering theories of recombination it is necessary to know whether factors which affect allelic recombination also affect inter-genic recombination. Consequently, experiments were made to measure the frequency of recombination between flanking marker genes in the general populations of some of those sets of crosses exhibiting significantly different frequencies of histidine prototrophs.

(i) *Frequency of allelic recombination and its relation to recombination between marker genes.* Estimates of the rate of recombination between flanking markers in the general population of a number of crosses were obtained in two ways. In the first, suspensions of ascospores were plated on Vogel's medium supplemented with histidine, uracil and leucine and resulting colonies were classified for their marker constitution. The second was a selective method in which suspensions of ascospores were plated on Vogel's medium supplemented with histidine. Only the recombinant class that is prototrophic with respect to the outside markers is able to grow on this medium. An estimate of the viability of the spores was obtained by plating on Vogel's medium containing histidine, uracil and leucine. The frequency of prototrophic recombinants was then determined and doubled to give the frequency of recombination between the marker genes.

Using the selective method, values for the frequency of recombination between flanking markers were obtained for three crosses between K540 and K516, three between K540 and K553, two between K548 and K553 and two between K553 and K516. The results are tabulated in table 3 which shows the limits to the frequency of recombination between the flanking markers and also the limits to the frequency of histidine prototroph formation when $P = 0.05$. Values for recombination between flanking markers using the non-selective method were obtained for two of the three crosses between the *his-5* alleles K540 and K516 and are shown in table 4. These values are almost identical to those observed for the same crosses using the selective method. The data in table 4 also indicate that the recombinant classes occur with equal frequency as is expected in crosses between genes.

In order to show that the differences in the frequencies of histidine prototroph formation and recombination between flanking markers are not the result of variations in environmental conditions that might affect recombination, two of the crosses between K540 and K516 were set up again after a considerable interval and fresh estimates for the frequencies obtained. The results of the repeated crosses are shown in table 3, serial numbers 29b and 31b. It is apparent that the estimates from the repeated crosses do not differ significantly from the original estimates and it therefore seems highly probable that the variations of the various frequencies in the independently or reciprocally marked crosses are genetic in origin.

Inspection of the data in table 3 reveals a correlation between frequency of histidine prototroph formation and frequency of recombination between flanking markers. The relationship is best understood if the results are shown graphically. Fig. 2 shows the frequency

TABLE 3

Showing per cent. recombination between proximal and distal markers and frequency of histidine prototroph formation. Limits to the frequencies are shown where $P = 0.05$. The isolate number of the components of each cross immediately succeed the mating type

Cross		Total germination	No. of +his+ colonies	Per cent. recombination between <i>pyr-3</i> and <i>leu-2</i>	Frequency of histidine prototrophs per 10^5 ascospores		
Serial no.	Marker constitution <i>pyr-3</i> $\times$ <i>leu-2</i>					Upper limit $P = 0.05$	Lower limit $P = 0.05$
29a	K ₅₄₀ <i>ai</i> $\times$ K ₅₁₆ <i>AI</i>	5247	245	9.34 ± 1.14	9.3	12.1	7.14
29b	K ₅₄₀ <i>ai</i> $\times$ K ₅₁₆ <i>AI</i>	1533	66	8.61 ± 2.35	9.6	11.6	7.9
30	K ₅₄₀ <i>ai</i> $\times$ K ₅₁₆ <i>A2</i>	4968	312	12.57 ± 1.35	13.3	14.9	11.8
31a	K ₅₁₆ <i>ai</i> $\times$ K ₅₄₀ <i>AI</i>	1681	156	18.56 ± 2.77	17.9	20.5	15.6
31b	K ₅₁₆ <i>ai</i> $\times$ K ₅₄₀ <i>AI</i>	2195	203	18.5 ± 2.54	16.5	19.7	13.7
56	K ₅₄₀ <i>ai</i> $\times$ K ₅₅₃ <i>A3</i>	5450	253	9.28 ± 1.12	2.2	2.53	1.83
57	K ₅₄₀ <i>ai</i> $\times$ K ₅₅₃ <i>A4</i>	5255	302	11.49 ± 1.26	2.6	3.09	2.14
55	K ₅₅₃ <i>ai</i> $\times$ K ₅₄₀ <i>AI</i>	1959	176	17.97 ± 2.53	3.8	4.33	3.31
27	K ₅₅₃ <i>ai</i> $\times$ K ₅₁₆ <i>A2</i>	2676	128	9.57 ± 1.62	4.8	5.6	3.99
26	K ₅₅₃ <i>ai</i> $\times$ K ₅₁₆ <i>AI</i>	1213	103	16.98 ± 3.14	6.4	7.6	5.37
60	K ₅₄₈ <i>AI</i> $\times$ K ₅₅₃ <i>a2</i>	1669	69	8.27 ± 1.91	13.1	15.3	11.3
62	K ₅₅₃ <i>ai</i> $\times$ K ₅₄₈ <i>A3</i>	1349	120	17.79 ± 3.04	16.0	18.4	13.8

TABLE 4

*Showing recombination per cent. between *pyr-3* and *leu-2*. Limits to the frequencies are given where $P = 0.05$*

Cross		Genotypes of progeny				Per cent. recombination between <i>pyr-3</i> and <i>leu-2</i>
Serial no.	Marker constitution <i>pyr-3</i> × <i>leu-2</i>	Parental combinations		Recombinant combinations		
		<i>pyr his</i> +	+ <i>his leu</i>	+ <i>his</i> +	<i>pyr his leu</i>	
29	K540 <i>ai</i> × K516 <i>AI</i>	179	196	18	22	9.64 ± 2.84
31	K516 <i>ai</i> × K540 <i>AI</i>	127	126	28	28	18.12 ± 4.3

(a)

A K540 1298 a1 X K516 37501 A 1
 B K540 1298 a1 X K516 37501 A 2
 C K516 1298 a1 X K540 37501 A 1

(b)

A K540 1298 a1 X K553 37501 A 3
 B K540 1298 a1 X K553 37501 A 4
 C K553 1298 a1 X K540 37501 A 1

(c)

A K548 1298 A 1 X K553 37501 a 2
 B K553 1298 a 1 X K548 37501 A 3

(d)

A K553 1298 a1 X K516 37501 A 2
 B K553 1298 a1 X K516 37501 A 1

FIG. 2.—The frequency of histidine prototroph formation plotted against the frequency of recombination between flanking markers for four sets of crosses between pairs of *his-5* alleles that are reciprocally or independently marked.

of histidine prototroph formation plotted against the frequency of recombination between flanking markers for crosses of the four types $K_{540} \times K_{516}$, $K_{540} \times K_{553}$, $K_{548} \times K_{553}$ and $K_{553} \times K_{516}$. In the crosses between K_{540} and K_{516} and K_{540} and K_{553} , fig. 1*a* and 1*b* respectively, the increases in frequency of allelic recombination are directly proportional to the increases in frequency of recombination between flanking markers. In the crosses between K_{548} and K_{553} and K_{553} and K_{516} , fig. 1*c* and 1*d* respectively, increases in the frequency of allelic recombination are accompanied by proportionally much larger increases in frequency of recombination between the flanking markers and the probability of the increases being proportional is less than 0.01 in each case.

(ii) *Mapping by prototroph frequencies.* A map of the fine structure of the *his-5* gene based on the frequency of prototroph formation in crosses between pairs of *his-5* alleles is shown in fig. 3. Where more than one value was obtained for the frequency of allelic recombination between a pair of alleles, the frequency shown is the weighted mean. The significant variation in frequency of prototroph formation already noted indicates that considerable caution is necessary in constructing the map. However, even when the alternative values for prototroph frequencies are substituted in the map, the order of the sites of difference between the alleles inferred from the data does not change.

In the reciprocally marked crosses between K_{78} and K_{155} no prototrophs were observed in a total of 64×10^5 viable ascospores. This might be thought to indicate that the two alleles are the result of changes at the same site in the genetic material. Unfortunately, the two mutants were both isolated in the same experiment, an examination of which reveals the distinct possibility that these mutants are two isolates of the same original colony.

Two crosses are outstanding for their unexpectedly low frequency of prototroph formation. These two are the crosses $K_{550} \times K_{542}$ and $K_{550} \times K_{729}$ which gave frequencies of 0.11 per 10^5 and 0.09 per 10^5 spores respectively whereas the expected values were in the region of 20 per 10^5 . The frequencies in these crosses are so low that they might be the result of reversion of one of their component stocks. Indeed the self cross $K_{550} \times K_{550}$ did indicate a higher frequency of reversion than is usual for other selfed *his-5* mutants. Further information on the reversion frequency of K_{550} and several other *his-5* mutants was obtained by plating large numbers of conidia in Vogel's medium and counting the histidine prototrophs that occurred. The results of these tests are shown in table 5. It is apparent that the frequency of K_{550} is of the order of twenty times that of the other four *his-5* mutants tested. The frequency of reversion amongst conidia of K_{550} , 1 in 4×10^6 conidia, although not directly comparable to the frequency of reversion in ascospores, indicates that an appreciable number of the histidine prototrophs found in the two crosses $K_{550} \times K_{542}$ and $K_{550} \times K_{729}$ could be the result of reversions of the

FIG. 3.—Genetic map of the *his-5* gene based on the frequency of histidine prototroph formation amongst ascospores from crosses between pairs of alleles. Distances are expressed as the frequency of prototrophs per 10^3 viable ascospores.

K₅₅₀ component. Thus it is quite possible that the rate of recombination between these pairs of alleles is considerably lower than the frequency of prototroph formation indicates. As yet no satisfactory explanation of these anomalous frequencies can be made.

(iii) *The additivity of prototroph frequencies.* It may be seen from the genetic map, fig. 3, that it is often found that the frequency of prototroph formation between a pair of alleles considerably exceeds the sum of the individual values for the intervals between the pair. It is convenient here to introduce the term map distance which may be defined as the sum of the individual frequencies of recombination between successive adjacent sites in the genetic map. If the additivity

TABLE 5

Spontaneous reversion to prototrophy amongst conidia

<i>His-5</i> allele tested	Number of viable conidia in millions	Number of reversions	Frequency of reversion per 10 ⁸ conidia	Limits to the frequency of reversion per 10 ⁸ conidia when $P = 0.05$	
				Lower limit	Upper limit
K ₅₅₀	21.7	5	23.0	7.47	53.8
K ₅₁₆	127.8	1	0.78	0.198	4.36
K ₅₄₀	258.3	4	1.55	0.422	3.96
K ₅₅₃	333.8	3	0.899	0.185	2.63
K ₇₂₉	234.6	3	1.23	0.264	3.74

rule as previously stated (p. 257) holds then the frequency of recombination between any pair of alleles should equal the map distance between them. Fig. 4 shows the frequency of recombination plotted against the map distance for each cross. It is apparent that the data generally indicate that the frequency of recombination exceeds the map distance and that the higher the recombination frequency the more it exceeds the map distance. This phenomenon is termed map expansion. Although the majority of the data indicates a considerable degree of map expansion there is a group of crosses that is exceptional in exhibiting a low degree of map expansion or in some cases a degree of map contraction. Almost all of these crosses have one parental component in common this being the *his-5* allele K₅₅₀. In fig. 4 all crosses in which one parental strain contains K₅₅₀ are distinctively marked with circles.

(b) *Distribution of flanking markers*

The distribution of the flanking markers *pyr-3* and *leu-2* amongst histidine prototrophs is shown in table 2 which also shows the order of the *his-5* allele differences as inferred according to the three criteria already explained (p. 3). The orders are expressed in terms of the

proximal allele difference. For each cross where more than ten prototrophs are concerned in the comparison made, an indication is given of the statistical reliability of the orders. Those significant at

FIG. 4.—The frequency of histidine prototroph formation in crosses between pairs of *his-5* alleles plotted against the map distance between them. Results of crosses in which one parental strain contains K550 are marked with circles.

the 5 per cent. level ($P = 0.05$ or less) are shown in bold type, while those with a P value between 0.05 and 0.3 are shown in italics. Standard type is used for those with a P value of 0.3 or greater and also where less than ten prototrophs are concerned in the comparison.

FIG. 5.—The order of the allele differences deduced from the distribution of flanking markers amongst the prototrophic recombinants formed in crosses between pairs of *his-5* alleles. Each horizontal line represents a cross and the arrowheads indicate the distal allele. (a) The best order obtained using criterion I. (b) The best order obtained using criterion II. (c) The best order obtained using criterion III.

As a whole, these data indicate an order of the allele differences (fig. 5) which is consistent with the order derived from comparison of prototroph frequencies (fig. 3). The consistency of the orders derived from considering the distribution of the flanking markers is most readily grasped by plotting the data by means of lines joining the positions of the allele differences, the distal position being marked by an arrow. In fig. 5, the orders significant at the 5 per cent. level are indicated by three arrows. Those orders where P is between 0.05 and 0.3 are indicated by two arrows and those where P exceeds 0.3 are indicated by a single arrow. Criteria I and II give a high degree of consistency, criterion III being somewhat less consistent. There is one notable exception to the consistency of the order of allele differences inferred by the three criteria and once again this concerns the *his-5* mutant K550. Criteria I and II both indicate that K550 is between K533 and K512 towards the distal end of the gene. This disagrees with its location at the opposite end of the gene in the genetic map. Criterion III differs from the other two criteria in locating K550 between K268 and K78 towards the proximal end of the gene. The location of K550 by criterion III is not significantly different from its location in the genetic map based on prototroph frequencies.

4. RELATION BETWEEN GENETIC AND COMPLEMENTATION MAPS

Ninety *his-5* mutants were tested, in all possible pairwise combinations, for their ability to complement one another. Fifty-eight of the mutants had been tested for complementation previously (Catcheside, 1960). With one exception the results for these mutants are identical to those previously reported. However, complementation could not be demonstrated between the alleles K516, complementation group B of Catcheside, and K541, group F, as was previously reported (Catcheside, 1960). Neither did K516 complement other new group F mutants. The combination K516+K541 was further studied by combining the *methionine-3* mutant 36104 with K516 and the *leu-2* mutant 37501 with K541 so that a forced heterocaryon cultured on minimal medium supplemented with histidine, could be prepared. In turn the heterocaryon was tested for its ability to grow on minimal medium, with the finding that it was unable to grow on this medium. It must be concluded that K516 does not complement K541, and the apparent complementation between them observed by Catcheside probably resulted from a reversion of one of the two mutants. K746, previously the sole member of group C, has now been included with K516 in group B since these two mutants exhibit the same complementation characteristics.

Of the 32 *his-5* mutants not previously tested for complementation only one, K877, exhibited a new complementation pattern. K877, now designated group C, complements only groups F and G mutants, a complementation pattern which fills a gap in the complementation

map, and which orders the groups more definitely in the complementation map. The ninety *his-5* mutants are listed in table 1 in their complementation groups, and the complementation pattern of the mutants has been interpreted in terms of a linear complementation map (fig. 6).

FIG. 6.—Comparison of genetic and complementation maps. Note that the two maps are not colinear.

FIG. 7.—Comparison of genetic and complementation maps. The order of the allele differences in the folded genetic map mimics the order of their complementation groups in the complementation map.

We are now in a position to compare the genetic and complementation maps. In fig. 6 it will be seen that the non-complementing mutants are scattered throughout the genetic map. The four group G mutants occur in two pairs which are separated by only a single group A mutant. The two group B mutants are closely linked proximally

to the group G mutants and the H and I mutants are closely linked and distal to the group G mutants. Thus it is clear that the two maps are not colinear. One way in which the order of the mutants in the genetic map can be made to mimic the order of their complementation groups in the complementation map is by folding the genetic map back on itself as shown in fig. 7. It should be noted that in order to preserve the scale of the genetic map the fold must be between the two pairs of group G mutants. Since it may be that the relation between the genetic and complementation maps reflects in some way the structure of the enzyme produced, it will be of great interest to see if the location of further complementing mutants in the genetic map supports the relation demonstrated so far.

5. DISCUSSION

The main purpose of the study of recombination between *his-5* mutants was to construct a genetic map to show the relative positions of the allelic differences, and to compare this with the complementation map. Since it has been demonstrated in other genes that complementation occurs at the protein level (Woodward, 1959; Fincham and Coddington, 1963) it is not unreasonable to assume that the position in the genetic map of the sites of difference of mutants of each complementation group may reflect in some way the structure and geometry of the enzyme produced. In the *his-5* gene the non-complementing mutants appear to be scattered throughout the length of the genetic map. This is also the case in *pan-2* (Case and Giles, 1958) and *me-2* (Murray, 1960, 1963) and seems to be the more usual arrangement, although in *his-1* (Jessop and Catcheside, unpub.) the non-complementing mutants are all concentrated at the proximal end of the map. *His-5* mutants of each complementation group appear to be grouped together in the genetic map although not in the same linear order as in the complementation map. However, since only a few complementing *his-5* alleles have been located in the genetic map the exact relation between the maps is not yet clear. Nevertheless, it is certain that the two maps are not entirely colinear.

The distribution of the flanking markers amongst prototrophic recombinants appears to provide a reliable indication of the order of the sites of difference in the *his-5* gene. Three criteria (Catcheside, Jessop and Smith, 1964) based on the distribution of the markers amongst the prototrophs of each cross when applied to the data systematically indicate, with remarkable consistency, an order for the sites of difference that agrees with their order in the genetic map based on the frequency of prototroph formation. These same criteria when applied to the *me-2* (Murray, 1960, 1963), *pan-2* (Case and Giles, 1958) and *his-1* data (Jessop and Catcheside, 1965) all indicate with reasonable consistency, orders for the sites of difference that are the same as those based on the frequency of prototroph formation. In other data the asymmetries of marker distribution are not always so

sharp but are usually shown to some extent by one of the flanking markers.

A reliable method of ordering allelic differences would be provided by mapping topologically, using a set of overlapping deficiencies. A topological map of the *his-5* gene may be expected to indicate the same order of the allelic differences as is indicated by the criteria already used. Efforts are now being made to induce and select structural mutants that may be suitable for constructing a topological map.

The only *his-5* allele that does not appear to behave as a point mutation is K550. In the genetic map based on prototroph frequencies K550 appears to be located at the proximal end of the map. However, two of the flanking marker criteria, criteria I and II, indicate a position towards the distal end of the map. In addition, when K550 was crossed with two alleles located in the centre of the map unexpectedly low frequencies of prototrophs were found. It seems possible that K550 is a structural mutation of some complexity although it cannot be a deletion since it has the highest reversion rate of all the *his-5* alleles tested. The high reversion rate of K550 also seems to indicate that it is not a double mutant. Nevertheless, a second mutant site located close to K542 and K729 would explain the low rates of recombination observed in crosses between K550 and these alleles. Even so it is difficult to see why low rates of recombination are not also shown in crosses between K550 and other alleles that are closely linked to K542 and K729. It is hoped that topological mapping will provide a more certain indication of the nature and location of this anomalous allele.

The differences in prototroph formation observed in certain crosses between the same pairs of *his-5* alleles that are reciprocally or independently marked are particularly interesting. An understanding of recombination at the molecular level is of fundamental importance but it is only recently that theories have been proposed which take account of the molecular structure of deoxyribonucleic acid and of its time of replication with respect to the time of genetic recombination at meiosis (Holliday, 1962, 1964; Whitehouse, 1963; Hastings and Whitehouse, 1964). These proposals include, variously, separation over a short distance of the two chains of a DNA molecule, the breakage of one or both of these separated chains, the formation of segments of DNA of hybrid origin, the excision of mispaired bases or segments of a DNA chain, and the reconstruction of segments of the DNA chain using information in the other chain. Many of these events would involve chemical reactions. It is a characteristic of organisms that such reactions are catalysed by enzymes and that each enzyme is structurally specified by a gene which may be controlled either directly or indirectly by other genes. It must be in these terms that the differences in frequency of recombination between various *his-5* stocks must be interpreted.

The most plausible suggestion that can be made is that the difference

in recombination frequencies is the result of some effect on the breakage process. Variation in the efficiency of an enzyme whose function is to produce breaks in DNA molecules would bring about variation in the frequency of the breaks and thus vary the number of occurrences of subsequent events that may lead to recombination. This would be a mechanism which would be expected to change the frequency of recombination without necessarily altering the distribution of the flanking markers amongst the prototrophic recombinants. In the cases observed, changes in frequency of recombination do not appear to be correlated with any changes in the distribution of the flanking markers. If recombination between alleles and non-alleles is by the same process then an increase in the effectiveness of an enzyme producing breaks should be coupled with proportional increases in allelic and non-allelic recombination as was found in the *his-5* data. It will also be appreciated that variations in efficiency of an enzyme producing breaks should affect allelic recombination at all loci and not just a single locus. However, adequate test of this may be frustrated by the considerable technical difficulty of measuring the relatively small differences in the frequency of recombination that are observed in the *his-5* data. If it is assumed that the higher frequencies observed in the *his-5* data are the result of an enzyme which is more efficient in producing breaks then it may be expected that the high frequency form will be dominant to the low frequency form. Since it seems probable that the differences in frequency observed are genetically controlled efforts are being made to test this prediction.

The remarkable feature of recombination data from various fungi is the great variability affecting all aspects of recombination which exists not only between different species, but also between genes in the same species and even between the same pairs of alleles. In the past, interest has been concentrated on fine structure mapping and on the nature of "conversion". In these respects, the variability observed has been regarded as more of a nuisance than a possible source of information about mechanisms (Catcheside, Jessop and Smith, 1964). It appears that close study of the nature and origin of genetic and other variations that affect recombination is essential to any complete understanding of the molecular processes that produce genetic recombination.

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6. REFERENCES

- AMES, B. N., AND HORECKER, B. L. 1956. The biosynthesis of histidine; Imidazole acetol phosphate transaminase. *J. Biol. Chem.*, 220, 113-128.
CASE, MARY E., AND GILES, N. H. 1958. Recombination mechanisms at the *pan-2* locus in *Neurospora crassa*. *Cold Spring Harb. Symp. Quant. Biol.*, 23, 119-135.

- CATCHESIDE, D. G. 1960. Complementation among histidine mutants of *Neurospora crassa*. *Proc. Roy. Soc. Lond.*, B153, 179-194.
- CATCHESIDE, D. G., JESSOP, ADRIENNE P., AND SMITH, B. R. 1964. Genetic controls of allelic recombination in *Neurospora*. *Nature*, 202, 1242-1243.
- FINCHAM, J. R. S., AND CODDINGTON, A. 1963. Complementation at the *am* locus of *Neurospora crassa*: a reaction between different mutant forms of glutamate dehydrogenase. *J. Mol. Biol.*, 6, 361-373.
- HOLLIDAY, R. 1962. Mutation and replication in *Ustilago maydis*. *Genet. Res.*, 3, 472-486.
- HOLLIDAY, R. 1964. A mechanism for gene conversion in fungi. *Genet. Res.*, 5, 282-303.
- JESSOP, ADRIENNE P., AND CATCHESIDE, D. G. 1965. Interallelic recombination at the *his-1* locus in *Neurospora crassa* and its genetic control. *Heredity*, 20, 237-256.
- MURRAY, NOREEN E. 1960. Complementation and recombination between methionine-2 alleles in *Neurospora crassa*. *Heredity*, 15, 207-217.
- MURRAY, NOREEN E. 1963. Polarised recombination and fine structure within the *me-2* gene of *Neurospora crassa*. *Genetics*, 48, 1163-1183.
- NEWMAYER, DOROTHY. 1954. A plating method for genetic analysis in *Neurospora*. *Genetics*, 39, 604-618.
- SMITH, B. R. 1962. Linkage data for group IV. *Neurospora Newsletter*, 1, 16-17.
- VOGEL, H. J. 1955. A convenient growth medium for *Neurospora*. *Microbial Genet. Bull.*, 13, 42.
- WESTERGAARD, M., AND MITCHELL, H. K. 1947. *Neurospora* V. A synthetic medium favouring sexual reproduction. *Amer. J. Bot.*, 34, 573-577.
- WHITEHOUSE, H. L. K. 1963. A theory of crossing over by means of hybrid deoxyribonucleic acid. *Nature*, 199, 1034-1040.
- WHITEHOUSE, H. L. K., AND HASTINGS, P. J. 1965. The analysis of genetic recombination on the polaron hybrid DNA model. *Genet. Res.* (in press).
- WOODWARD, D. O. 1959. Enzyme complementation *in vitro* between adenylosuccinaseless mutants of *Neurospora crassa*. *Proc. nat. Acad. Sci., Wash.*, 45, 846.