INTERALLELIC RECOMBINATION AT THE *his-1* LOCUS IN *NEUROSPORA CRASSA* AND ITS GENETIC CONTROL*

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Received 17.xi.64

1. INTRODUCTION

THE *histidine-1* gene of *Neurospora crassa* is situated in the right arm of chromosome V between *amination* and *inositol* (Strickland, Perkins and Veatch, 1959). It apparently controls the primary structure of the enzyme imidazole glycerol phosphate dehydrase, which catalyses the conversion of imidazole glycerol phosphate to imidazole acetol phosphate in the biosynthesis of histidine (Ames, 1960).

A large number of *his-1* mutants are known and some of these exhibit complementation. Initially the complementation matrix was thought to indicate a two-dimensional complementation map (Catchside, 1960), but this is now known to be an error (see pp. 17-19). Nevertheless, this was the reason for attempting to map the fine structure of *his-1*. Such mapping depends primarily upon the frequency of prototrophs found amongst ascospores produced by repulsion phase crosses between pairs of auxotrophs. It assumes that the frequency of prototrophs formed by such a cross is a measure of the physical distance between the actual sites at which the two auxotrophs differ from one another and so from the wild type gene. In consequence, it is expected that a series of allelic auxotrophs, all with point differences, would show prototroph frequencies that conformed to an additivity rule, namely that the frequency shown by a cross between any two auxotrophs would be the sum or the difference of the frequencies shown by crosses of these two auxotrophs with a third auxotroph. If this rule held and the frequencies were measured sufficiently accurately, the sites of mutation should be capable of arrangement in an unambiguous linear order.

However, two features of the *his-1* data on prototroph frequencies led to difficulties. Firstly, many of the frequencies were very low and, secondly, as was later found, crosses involving different stocks of the same auxotrophs commonly gave widely different values. With further study, it became clear that the different values were mainly due to the

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effects of genetic differences, principally at a locus designated *rec*, for recombination. Crosses, it was found, could be divided phenotypically into those with high and those with low frequencies of prototrophs, the factor of difference being about ten. Genotypically, the observations are explicable by assuming two allelic genes, *rec* and *rec*⁺, such that only crosses in which both parents were *rec* gave high frequencies of prototrophs, while crosses in which one or both of the parents were *rec*⁺ gave low frequencies. In brief, the gene for low frequency is dominant to that for high frequency. Most of the stocks used may, with some confidence, be classified as *rec* or *rec*⁺.

Starting with a set of *his-1* mutants, all derived from one wild-type strain, crosses between them could be made only after outcrossing to introduce the other mating type, while further outcrosses were necessary to introduce the flanking markers. In these various manœuvres, the source and spread of so cryptic a genetic difference as the one described above will be hard to trace completely.

From the beginning of the work, reliance for mapping was not solely dependent upon prototroph frequency, but also on the distribution of the combinations of flanking markers, proximal and distal, amongst the prototrophs produced by crosses. If in a cross of the general type $Am^1B \times am^2b$, where m^1 and m^2 are alleles whose recombination is being studied, while *A* and *a* and *B* and *b* are, respectively, the proximal and distal flanking markers, selection is made for m^+ prototrophs, the flanking markers occur in four combinations. These are the two parental combinations *AB* and *ab* and the two recombinants *Ab* and *aB*. In *Neurospora* all four classes occur with substantial frequencies, where recombination of alleles is studied. A vast excess of one recombinant class, so characteristic of the distribution of flanking markers when prototrophic recombinants of two closely linked non-allelic genes are selected and indicative of the order of the gene differences, is not encountered. Nevertheless, the data do show some regularities. The parental classes are often unequal in size. The recombinant classes are often unequal in size. The directions of inequality of the parental and recombinant classes are correlated. The inequalities are usually not very great. It has been customary to assume that the high frequencies of the parental and the second recombinant classes are due to the conjoint occurrence of two or three crossovers showing a high incidence of negative interference. This is no more than a description of what the observations require in order to fit them to the chiasmotype theory of genetic recombination. We are not concerned here so much with the theory of recombination, as with what information about order of allelic differences may be extracted from the data.

It is simplest to understand the criteria, if it is assumed that the recombination does occur by a process analogous to crossing-over (fig. 1). Three relationships 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.

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; they entered the cross together.

3. If the distal flanking alleles only 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; 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 of the progeny scored.

FIG. 1.—Diagrams to show the derivation of the criteria of order based upon the distribution of flanking genetic markers (A and B) amongst prototrophs formed by allelic auxotrophs (m^1 and m^2).

If any, or all, of these criteria lead to the derivation of a consistent order of a series of m alleles, this order may be regarded as acceptable and meaningful. Consistency may be expected to hold between the three criteria applied to one pair of alleles and between a series of pairs of a set of alleles considered with respect to one criterion. Inconsistency can be considered significant only if the order inferred is based upon a significant difference in frequency between the classes compared. The *his-1* data, as will be seen, mostly show rather small frequency differences, which are insignificant and therefore ambiguous.

It is convenient to distinguish between the two parental classes (P_1 and P_2) and the two recombinant classes (R_1 and R_2) in a perfectly general way. P_1 may be defined as the parental class which has the flanking markers which went into the cross with the proximal allele difference. R_1 may be defined as the recombinant class which combines the proximal flanking marker of the distal allele difference with the distal flanking marker of the proximal allele difference. Thus in the cross $Am^1+B \times a+m^2b$, the P_1 progeny are AB and the R_1 progeny are aB .

2. MATERIALS AND METHODS

(a) Organisms

The majority of the *his-1* mutants (all those with a number prefixed by the letter K) were isolated from Emerson a by filtration enrichment following irradiation of

TABLE 1

Cross		<i>his-1</i> prototrophs					Proximal allele difference by criterion		
Serial no.	Constitution	Number per 10 ⁵ ascospores	Flanking markers				I	II	III
	<i>am</i> parent <i>inos</i> parent		<i>am</i>	<i>inos</i>	++	<i>am inos</i>			
A <i>rec</i> × <i>rec</i>									
1	K83(1) × K90	17.3	58	55	49	35	K83	(K83)	K83
*2	K83 × K90	17.7	37**	30	31	18†	K83	(K83)	K83
3	K83(1) × K141	45.7	41	39	35	22	K83	K83	K83
4	K83(1) × K566(2)	4.2	42	45	35	22	K83	K83	(K83)
*5	K83 × K616(1)	64.2	—	67	107	—	—	—	K83
6	K83(1) × K616(1)	41.9	51	22	39	24	K83	K616	K83
7	K83(1) × K627	4.3	41	40	37	17	K83	K83	K83
8	K83(1) × K651	30.2	36	24	33	19	K83	(K83)	K83
9	K83(6) × K651	26.5	26	39	42	14	K83	K83	K83
10	K83(9) × K651	32.9	24	29	24	18	(K83)	K83	(K83)
11	K83(10) × K651	30.7	14	8	6	4	(K83)	(K651)	K83
12	K83(1) × Y189-M89	11.7	70	37	55	27	K83	(Y189-M89)	K83
13	K627(2) × K83	2.9	26	20	22	15	K627	(K627)	K627
14	K627(2) × K616(1)	49.9	69	36	54	21	K627	=	K627
15	K627(2) × K651	31.8	70	40	47	40	(K627)	K651	K627
16	K627(2) × Y189-M89	11.3	54	27	39	15	K627	(Y189-M89)	K627
17	K651 × K83	25.0	115	114	72	105	K83	K83	K83
18	K651 × K566(2)	36.7	24	51	15	22	K566	K651	K566
19	K651 × K616(1)	17.3	30	5	23	18	(K651)	K616	K651
20	K651 × K627(2)	42.1	45	77	26	39	K627	K651	K627
21	Y189-M89(2) × K83	17.3	64	94	42	72	K83	(K83)	K83
22	Y189-M89(2) × K566(2)	19.0	23	38	14	29	K566	=	K566
23	Y189-M89(2) × K627(2)	23.6	34	69	40	39	(Y189-M89)	Y189-M89	K627
B <i>rec</i> × <i>rec</i> ⁺									
*24	K83 × K616(2)	4.0	9**	17	26	13†	K83	K83	(K83)
25	K83(1) × K625	3.1	10	24	28	15	K83	K83	(K625)
26	K83(1) × K742(1)	0							
27	K627(2) × K742(1)	0							
28	Y189-M89(2) × K742(1)	0							
C <i>rec</i> × ?									
29	K83(1) × K85	0.64	3	8	4	1	K83	K83	(K85)
30	K83(1) × K245	5.6	12	25	17	14	(K83)	K83	K245
31	K627(2) × K649	0.61	2	6	0	0			
32	K627(2) × C84	0.59	1	0	0	2			
D <i>rec</i> ⁺ × <i>rec</i>									
33	K83(2) × K90	1.63	5	4	6	0	K83	K83	K83
34	K83(2) × K141	2.76	2	19	16	9	K83	K83	K141
35	K83(2) × K566(2)	0.19	1	0	0	0	—	—	—
36	K83(2) × K616(1)	3.91	10	9	7	6	(K83)	=	(K83)
37	K83(2) × K627(2)	0.29							
38	K83(2) × K651	1.73	16	14	11	7	(K83)	(K83)	(K83)

Notes.—(i) All families, except those marked with an asterisk, are of the general form *am his-1*[±] × *his-1*[±] *inos*. (ii) Families 5, 46, 47, 55, 56, 57, 58, 62, 65 and 67 are of the general form *his-1*[±] × *his-1*[±] *inos*. (iii) Families 2 and 24 are of the general form *sp his-1*[±] × *his-1*[±] *inos*. (iv) Where two or more stocks of a given constitution have been used in the crosses, these are distinguished by bracketed numbers placed after the *his-1* allele number. (v) Constitution of

TABLE 1—(continued)

Cross		<i>his-1</i> prototrophs					Proximal allele difference by criterion		
Serial no.	Constitution	Number per 10 ⁵ ascospores	Flanking markers				I	II	III
	<i>am</i> parent <i>inos</i> parent		<i>am</i>	<i>inos</i>	++	<i>am inos</i>			
39	K83(4) × K651	1·34	1	1	1	2			
40	K83(5) × K651	2·95	2	3	2	5	—	(K651)	K651
41	K83(7) × K651	2·91	2	0	3	6	—	K651	(K651)
42	K83(8) × K651	1·05	1	6	1	0			
43	K83(11) × K651	2·63	1	1	1	1			
44	K83(12) × K651	2·57	4	4	2	1	—	(K83)	(K83)
45	K83(13) × K651	2·45	1	4	5	1	—	K83	(K83)
*46	K85 × K616(1)	0·79	—	12	8	—	—	—	(K616)
*47	K85 × K627(2)	3·84	—	14	9	—	—	—	K627
48	K617 × K83	0·5	2	1	3	3			
49	K617 × K141	3·3	10	29	33	14	K617	K617	=
50	K617 × K566(2)	0·24	4	0	1	0			
51	K617 × K627(2)	0·11							
52	K617 × K651	2·95	4	10	4	7	(K651)	(K617)	K651
53	K620 × K83	1·3							
54	K620 × K651	2·36	7	6	0	8	K651	K651	K651
*55	K625 × K616(1)	0							
*56	K625 × K627(2)	8·63	—	14	15	—	—	—	(K625)
*57	K629 × K566(2)	0·82	—	8	6	—	—	—	(K566)
*58	K629 × K616(1)	3·21	—	49	54	—	—	—	(K629)
59	K742(2) × K83	0·05							
60	K742(1) × Y189-M89	0·59	9	14	13	0	K742	K742	K742
61	K744(1) × K83	1·0	3	3	4	4	—	=	=
E ? × <i>rec</i>									
*62	K651 × K141	1·3	—	10	4	—	—	—	K141
*63	Y189-M89(1) × K90	0							
F <i>rec</i> ⁺ × <i>rec</i> ⁺									
64	K83(2) × K625	3·70	6	25	6	10	K625	K83	K625
*65	K141 × K616(1)	0							
66	K617 × K625	3·23	9	23	18	7	K617	K617	(K625)
*67	K651 × K625	1·17	—	10	4	—	—	—	K625
G <i>rec</i> ⁺ × ?									
68	K617 × K85	1·70	0	12	3	0	—	—	K85
69	K620(1) × K629	0·44	3	5	2	1	—	(K620)	(K629)
H ? × <i>rec</i> ⁺									
70	K627(1) × K616(2)	1·1							
I ? × ?									
71	K83(3) × K620	0·06							
72	K620(1) × K616(3)	3·05	3	2	1	1			
73	K620(1) × K742(2)	0·38	0	0	1	0			
74	K627(1) × K566(1)	0							
75	K627(1) × K620	0							
76	K744(2) × K624	0·06	0	0	0	1			
77	K744(2) × K627(1)	0·1	1	1	1	0			

progeny marked * is *sp*, that of those marked † is *sp inos*. (vi) Amongst the prototrophs, the P₁ and R₁ flanking marker constitutions are identified by the numbers of progeny being in italics. (vii) The levels of significance of identification of the proximal allele difference by criteria I, II and III are indicated by bold face type for P less than 0·05, while those with P 0·3 or greater are bracketed.

conidia with ultra-violet light. These have been listed by Catcheside (1960). Stocks of two other *his-1* mutants, C84 and Y189-M89, were received from Dr B. Ames and Prof. N. H. Giles, respectively. The *amination* mutant used was K134, isolated by Catcheside from Emerson a; this is the allele referred to by Dr J. R. S. Fincham as *am*⁶. The *inositol* mutant used was 37401, originally received from Dr G. W. Beadle, while *spray* (B132) was donated by Dr B. R. Smith. The only other stocks which have entered the pedigree are the wild types Emerson A and Emerson a.

(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. Tests of cultures for biochemical differences were made on Vogel's medium in which 0.5 per cent. sorbose and 0.1 per cent. sucrose replaced the usual 2 per cent. sucrose; suitable supplements were added.

Appropriate supplements of growth factors were added to the minimal media as follows. For *his-1* and *his-1 inos* stocks, histidine was added at the rate of 300 mg./l. and inositol at 20 mg./l. For *am his-1* and *am* stocks, histidine was used at the rate of 200 mg./l. and alanine at 450 mg./l. *Amination* mutants will grow on a number of different amino acids and their growth is inhibited by some others (Fincham, 1950). Histidine is inhibitory, so the amount of it was reduced in media for *am his-1* stocks. However, *am* mutants can adapt to grow on minimal medium, so glycine at the rate of 1500 mg./l. was added to the medium when *am* was being scored.

(c) Crosses

Each cross for analysis of prototroph frequency was made in about eight tubes of crossing medium and the ascospores from the different tubes were pooled. Ascospores were collected in sterile water, filtered through butter muslin and, after decantation of excess water, 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. samples on slides on which the area covered by the coverslip was divided into 64 squares. The ascospores were screened for prototrophs by a modification of the layer plate technique (Newmeyer, 1954; Murray, 1960). The ascospores in the suspension in 0.1 per cent. agar were activated to germinate by heating at 55° C. for 60 minutes. Known volumes were then pipetted into 2 ml. volumes of 0.5 per cent. agar, kept molten at 45° C., quickly mixed and evenly spread on plates of Vogel's medium supplemented with inositol and alanine. The plates were incubated at 25° C. and scanned under a stereomicroscope after 24 hours and 48 hours. Prototrophs were transferred to tubes of Vogel's medium, containing inositol and alanine, and later classified for *am* and *inos*. The viability of the ascospores of each cross was determined in samples layer plated on Vogel's medium plus histidine, inositol and alanine.

3. RESULTS

When doubly mutant stocks had been prepared, so far as possible in both mating types, crosses of the type *am his-1*^x + × + *his-1*^y *inos*, where *his-1*^x and *his-1*^y designate two *his-1* alleles, were analysed for their frequency of formation of prototrophs and the prototrophs were then classified for the four combinations of flanking markers, two parental, *am* + and + *inos*, and two recombinant, ++ and *am inos*. The mutant *spray* was used as proximal marker in two crosses. In a number of crosses there was no proximal marker. The data are summarised in table 1, the important parameters in which are (i) the

frequencies of prototrophs per hundred thousand ascospores analysed in a cross and (ii) the order of the *his-1* allele differences as deduced from the distribution of the four classes of the flanking marker combinations amongst these prototrophs. These two aspects of the data will be discussed separately.

(a) *Prototroph frequencies*

The observed frequencies of prototrophs ranged from 0.1 (or even zero) to 50 or so per 10^5 ascospores. Of great interest and, initially, a

TABLE 2

Data on prototrophs in crosses of various his-1 inos A stocks to two stocks of am K83 a

<i>his-1 inos</i> A parent	<i>am</i> K83 a(1)					<i>am</i> K83 a(2)				
	No. per 10 ⁵ ascospores	Flanking markers				No. per 10 ⁵ ascospores	Flanking markers			
		<i>am</i>	<i>inos</i>	++	<i>am inos</i>		<i>am</i>	<i>inos</i>	++	<i>am inos</i>
K566 (2)	4.2	42	45	35	22	0.2	1	0	0	0
K627 (2)	4.3	41	40	37	9	0.3	—	—	—	—
K90	17.3	58	55	49	35	1.6	5	4	6	0
K141	45.7	41	39	87	31	2.76	2	19	16	9
K616 (1)	41.9	51	22	39	24	3.9	10	9	7	6
K651	27.0	36	24	33	19	1.7	16	14	11	7
K625	3.1	10	24	28	15	3.7	6	28	6	10
K83	0	—	—	—	—	0	—	—	—	—

source of embarrassment, were crosses involving different stocks of the same *his-1* mutant which gave substantially different frequencies of prototrophs. The analysis had best begin with a pair of stocks of one of these mutants, K83.

(i) *Genetics of prototroph frequency.* Two isolates of *am K83*,* both of mating type a, were obtained from a cross of $+K83 \text{ a} \times \text{am} +A$. These had the pedigree family numbers 2768 and 2769 and may be designated as *am K83(1)* and *am K83(2)*, respectively. Data on the progeny of crosses of both to a series of *his-1 inos A* stocks are gathered together in table 2. It will be noticed in all cases except one, namely the cross with K625 *inos*, that *am K83(1)* gives higher prototroph frequencies than does *am K83(2)*, by a factor of the order of ten. Unfortunately, the cross of *am K83(2)* with K83 *inos* was too infertile to give assurance that the *his-1* alleles of families 2768 and 2769 were indeed both K83. However, the data of table 2 would be difficult to explain on the assumption that, say, the *his-1* allele in family 2769 was not K83, for in this case the prototroph frequencies of the series of

* In writing the constitutions, only the allele numbers of the *his-1* mutants will be given. Strictly, the constitution here should be written $\text{am}^2 \text{his-1K83}$, but since only one mutant allele of *am* has been employed, the simpler form of the genotype is unambiguous.

crosses would not be proportional to those observed in the same series of crosses with family 2768.

No direct test for allelism was possible since families 2768 and 2769 are of the same mating type. Each was crossed to the wild stock Emerson A and a number of reisolates of *am his-1* were obtained. The *am his-1* reisolates could be of *a* or *A* mating type and by this indirect way the *his-1* alleles in families 2768 and 2769 would have been tested for allelism, had the crosses been sufficiently fertile. Isolates from family 2769, of *a* mating type, were also tested by crossing to K651

TABLE 3

his-1 prototrophs in crosses of K651 *inos A* with progeny of
am K83a(2) × Emerson A

<i>am K83a</i> isolate	<i>his-1</i> prototrophs				
	No. per 10 ⁵ ascospores	Flanking markers			
		<i>am</i>	<i>inos</i>	++	<i>am inos</i>
(4)	1.3	1	1	1	2
(5)	2.95	2	3	2	5
(7)	2.9	2	0	3	6
(8)	1.1	1	6	1	0
(11)	2.6	1	1	1	1
(12)	2.6	4	4	2	1
(13)	2.5	1	4	5	1
(6)	26.5	26	39	42	14
(9)	32.9	24	29	24	18
(10)	30.7	14	8	6	4

inos. In this way it was found that the progeny of *am K83 a(2) × Emerson A* were showing segregation for frequency of formation of prototrophs (table 3), seven giving low values and three high values, the latter being similar to those given by *am K83 a(1) × K651 inos A*. It therefore seems clear that a gene difference controlling prototroph frequency is segregating, that presence of the low frequency gene in one parent is sufficient to give low frequencies of prototrophs, while the presence of the high frequency gene in both parents is necessary to give high frequencies of prototrophs. If the locus concerned is designated *recombination*, the dominant allele for low frequency may be regarded as the wild type and the recessive allele for high frequency as mutant. Respectively they may be symbolised as *rec*⁺ and *rec*.

The *rec* gene has a large effect upon recombination between *his-1* alleles. Does it affect recombination between non-alleles or the distribution of flanking markers amongst *his-1* prototrophs? Does it influence other loci in a similar way? Work to answer the last question is in progress; it would, no doubt, be surprising if the effects

of *rec* were restricted to the *his-1* locus, but no answer is presently available.

Ascospores from crosses of *am* K83 *rec* and *am* K83 *rec*⁺ to *his-1 inos rec* testers have been analysed for recombination of *am* and *inos* (table 4). They were plated out on minimal medium, supplemented

TABLE 4

Parents	Progeny					Recombination per cent.	Difference	
	Total	<i>am</i>	<i>inos</i>	++	<i>am inos</i>		χ^2	P
<i>am</i> K83 × K651 <i>inos</i>								
(1) <i>rec</i> × <i>rec</i>	503	232	218	30	23	10.54	0.297	0.7-0.5
(2) <i>rec</i> ⁺ × <i>rec</i>	631	295	276	23	37	9.51		
<i>am</i> K83 × K616 <i>inos</i> (1)								
(1) <i>rec</i> × <i>rec</i>	902	410	405	40	47	9.56	1.428	0.3-0.2
(2) <i>rec</i> ⁺ × <i>rec</i>	798	372	370	27	29	7.02		

with histidine, alanine and inositol. This gave unselected populations, all members of which carried one of the *his-1* alleles of the parents. No attempt was made to classify the progeny for the *his-1* allele, but all were classified for *am* and *inos*. The *rec*⁺ × *rec* progenies show slightly smaller frequencies than do the corresponding *rec* × *rec* progenies, but the differences are not at all significant.

TABLE 5

Distribution of flanking markers in his-1⁺ progeny of rec × rec and rec⁺ × rec crosses of K83 and K651.

Cross	<i>am</i>	<i>inos</i>	++	<i>am inos</i>	Total
<i>am</i> K83 <i>rec</i> ⁺ × K651 <i>inos rec</i>	28	33	26	23	110
<i>am</i> K83 <i>rec</i> × K651 <i>inos rec</i>	100	100	105	55	360
<i>am</i> K651 <i>rec</i> × K83 <i>inos rec</i>	115	114	72	105	406

Data on the distribution of flanking markers amongst selected *his-1* prototrophs are contained in tables 1, 2 and 3. In general the data for the *rec*⁺ × *rec* crosses are too meagre to permit reliable comparison. Such differences as are shown in table 2 are inconsistent from one pair of crosses to another and similar differences are shown (table 3) in the series of *am* K83 *rec*⁺ crosses to K651 *inos rec*. The data of the K83 × K651 crosses are summed in table 5. The *rec* × *rec* crosses are seen to show significant asymmetries, while the *rec*⁺ × *rec* crosses do not. However, more data are needed before this can be accepted as a reasonable generalisation.

(ii) *Classification of stocks for rec.* It has been shown that the stocks so far considered may be classified for *rec*. Using them, attempts may be made to identify the constitutions of further stocks, with a view to grouping the prototroph data into high and low frequency sets. Besides *am* K83(2), which is *rec*⁺, K625 *inos* is the only other *rec*⁺ stock amongst those listed in table 2, all the rest being *rec*. These, then, provide a set of testers of known constitution with respect to recombination.

Strictly, to conclude whether a given stock of a *his-1* mutant is *rec*⁺ or *rec* requires measurement of the prototroph frequencies in crosses of it to known *rec*⁺ and *rec* stocks, carrying another suitable *his-1* allele, used as testers. A *rec*⁺ stock of *his-1*^x would give equal, low values of prototroph frequency with *his-1*^y *rec*⁺ and *his-1*^y *rec* testers. A *rec* stock of *his-1*^x would give different values with the testers; the value would be relatively low with *his-1*^y *rec*⁺ and relatively high with *his-1*^y *rec*. In general, such data are not available, because a sufficient range of pairs of tester stocks has not so far been included in the crosses. However, it is worth while to attempt to deduce the constitutions of the stocks, with the help of collateral evidence, so far as is possible.

Of course, Emerson A is *rec*, since segregation is shown in its cross with the low frequency stock *am* K83 a(2). The two stocks *am* K627(2) and K83 *inos* are both *rec* because their cross gives a frequency of prototrophs comparable to that of *am* K83(1) × K627 *inos* (2). Likewise, K83 is *rec*, because its cross gives a high frequency with K616 *inos* (1). Indeed the frequency is higher than in the cross *am* K83(1) × K616 *inos* (1), namely 64.2 ± 4.74 per 10^5 compared with 41.9 ± 3.23 per 10^5 . The difference, 22.3 ± 5.74 , is highly significant ($P < 0.001$), indicating that other factors, perhaps of genetic origin, influence prototroph frequencies, though in a less spectacular way than does *rec*.

The stocks *am* Y189-M89(2) and Y189-M89 *inos* must be *rec*. Since the cross *am* Y189-M89(2) × K90 *inos* gave a zero frequency of prototrophs, the sites of Y189-M89 and K90 must be very close together. However, the prototroph frequencies in the crosses *am* K83(1) × Y189-M89 *inos* and *am* Y189-M89(2) × K83 *inos* are both similar to the frequency in *am* K83(1) × K90 *inos*. By a comparable argument *am* K651 also appears to be *rec*. In particular, it gives similar prototroph frequencies in crosses to K83 *inos* and K627 *inos* (2), respectively, as does the known *rec* stock K651 *inos* with known *rec* stocks of K83 and K627. The stock *sp* K83 is also *rec*.

The above identifications probably exhaust all of the *rec* stocks in the data of table 1. Of course, reliance cannot be placed on the actual frequency of prototrophs in an isolated cross, since even two *rec* parents may have sites of difference so close together that recombination is rare. The observed range of prototroph frequencies in *rec* × *rec* crosses extends down to about 3 per 10^5 , while in *rec*⁺ × *rec* crosses it

extends up to about 4 per 10^5 according to the data in table 2. The lower values in *rec* $\times$ *rec* crosses overlap with the higher values in *rec*⁺ $\times$ *rec* and *rec*⁺ $\times$ *rec*⁺ crosses. Theoretically, the whole range to be found in *rec*⁺ crosses should be included in the lower tenth or so of the whole range to be found in *rec* $\times$ *rec* crosses.

Therefore, one indirect, though not perfectly conclusive, method of recognising a *rec*⁺ stock would be by the apparent compression of the range shown in crosses of it to *rec* stocks known to have *his-1* mutants well dispersed in the range. By this means, one may conclude that the following stocks are *rec*⁺: *am* K617, *am* K620(2), K620 *inos*, *am* K742 (1 and 2), K742 *inos* (1), K629, K85, K625 and K141. There is much less confidence in the suggestion that K85 *inos*, K629 *inos*, K651 and *am* K744(1) are also *rec*⁺. Nothing very definite can be said about the few remaining stocks used in a few crosses. In fact, only three of the *his-1* mutants were definitely used in both *rec* and *rec*⁺ stocks. These are K83, K141 and K616.

Twenty-four combinations of *amination* with eight different *his-1* mutants have been made. Omitting the ten stocks of *am* K83 isolated from *am* K83(2) $\times$ Emerson A, there are 4 *rec*, 6 *rec*⁺ and 4 uncertain; including the ten *am* K83 isolates there are 7 *rec*, 13 *rec*⁺ and 4 uncertain. Similarly, there are twenty-two combinations of *inos* with seventeen *his-1* mutants; 8 are *rec*, 4 are *rec*⁺, 2 more are probably *rec*⁺ and 8 cannot be determined. Of the stocks of *his-1* mutants used unaccompanied by *am* or *inos*, 4 are definitely *rec*⁺, one is probably *rec*⁺ and one is *rec*. The last of these, K83, was not the original mutant isolated from Emerson a, but a reisolate from the original stock crossed to Emerson A. It, therefore, appears highly probable that Emerson a carries the gene *rec*⁺, that all the mutants originally carried *rec*⁺ and that the introduction of *rec* has occurred by outcrossing.

Having identified the constitutions of the parental stocks with respect to *rec*⁺ and *rec* the set of 77 crosses available may be arranged in groups of high and low frequency crosses, as has in fact been done in table 1. Group A includes all of the crosses giving high frequencies of prototrophs. Groups B, D and F include the crosses giving low frequencies. Groups C, E, G, H and I include all the crosses in which one or both of the parents is of unknown constitution with respect to *rec*; these cannot be used in mapping. In general, within the high and low groups, respectively, the prototroph frequencies shown by crosses between different stocks of the same pair of *his-1* alleles are similar and only sometimes significantly different. Thus, crosses 8, 9, 10 and 11 involving K83 $\times$ K651 are consistent, not even the difference of 6.5 ± 3.95 between the least and greatest (crosses 9 and 10) being significant. Neither is the difference of 7.9 ± 3.88 between families 10 and 18 significant. Apart from the case of crosses 5 and 6 previously noted (p. 246), the only significant differences involve crosses 16 and 21 (difference 10.3 ± 3.78 , $P < 0.01$) and crosses 12 and 22 (difference 5.6 ± 1.33 , $P < 0.0001$).

(iii) *Mapping by prototroph frequencies.* All the data for each pair of *his-1* alleles may be combined, separately for the high and low frequency crosses, but omitting those crosses involving stocks whose *rec* constitution is uncertain or unknown. The estimates of prototroph

TABLE 6

Estimates of prototroph frequencies, per 10⁵ ascospores, in his-1 crosses

<i>his-1</i> alleles crossed	High frequency crosses			Low frequency crosses		
K83 × K90	17.4	±	0.91	1.61	±	0.42
K83 × K141	45.7	±	3.35	2.76	±	0.36
K83 × K566	4.2	±	0.32	0.19		
K83 × K616	51.3	±	2.73	3.96	±	0.40
K83 × K617				0.5		
K83 × K620				0.15		
K83 × K625				3.30	±	0.28
K83 × K627	3.61	±	0.23	0.29		
K83 × K651	27.0	±	0.95	1.93	±	0.18
K83 × K742				0.02		
K83 × K744				1.0	±	0.27
K83 × Y189-M89	14.5	±	0.66			
K85 × K616				0.78	±	0.17
K85 × K627				3.83	±	0.80
K90 × Y189-M89	0					
K141 × K616				0		
K141 × K617				3.3	±	0.35
K566 × K617				0.24		
K566 × K627	0					
K566 × K629				0.82	±	0.21
K566 × K651	36.7	±	3.39			
K566 × Y189-M89	23.6	±	1.74			
K616 × K620				3.05		
K616 × K625				0		
K616 × K627	49.9	±	3.61	1.1		
K616 × K629				3.21	±	0.31
K616 × K651	17.3	±	1.98			
K617 × K625				3.23	±	0.42
K617 × K627				0.11		
K617 × K651				2.95	±	0.59
K620 × K627				0		
K620 × K651				2.36	±	0.53
K620 × K742				0.38		
K625 × K627				8.63	±	1.60
K625 × K651				1.17	±	0.31
K627 × K651	36.0	±	1.82			
K627 × K742				0		
K627 × K744				0.1		
K627 × Y189-M89	16.05	±	0.90			
K742 × Y189-M89				0.49	±	0.08

frequencies, which may be used for the construction of a fine structure map, are summarised in table 6. Maps based on the high and low frequency data are shown in fig. 2. It will be seen that the two maps agree reasonably well, in respect of the order of the allelic differences and their relative spacing. However, the scales of the maps are greatly different, by a factor of about 14.5, rather than the tenfold difference as it has been described roughly in earlier paragraphs.

(b) Distribution of flanking markers

The data upon the distribution of the flanking markers, generally *am* and *inos*, are listed in table 1, which also contains the order of the *his-1* allele differences as inferred according to the three criteria explained in the introduction (p. 4). The orders are expressed in terms of the proximal allele difference, for every case where at least

FIG. 2.—Genetic maps of the *his-1* gene based on prototroph frequencies in progeny of crosses; (a) high frequency crosses, (b) low frequency crosses. The letters below the maps designate the complementation group to which the individual alleles belong.

ten prototrophs are concerned in the comparison made. For each entry, some indication is given of the statistical reliability of the orders. Those significant at the 5 per cent. level ($P = 0.05$ or less) are shown in bold type, while those with a P value of 0.3 or greater are enclosed in brackets. The P_1 and R_1 classes are identified in columns 6-9 of table 1 by italics; P_1 occurs in either the *am* or *inos* columns and R_1 in either the ++ or *am inos* columns.

Taken as a whole, these data indicate an order of the allele differences (fig. 3) which is consistent with the order derived from comparison of prototroph frequencies (fig. 2). The consistency of the order derived from consideration of 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 proximal position being marked by an arrow. In fig. 3, only the orders significant at the 5 per cent. level are used and the order used is that which gives the least number of discrepancies. Criterion I gives a high degree of consistency and this is maintained even if the orders based on less significant data are also included. The other two criteria are less consistent, criterion III giving better agreement than does criterion II.

(c) Comparison of genetic and complementation maps

Reference has already been made to the supposition that the complementation map of *his-1* may be two-dimensional (Catcheside,

1960). However, it was a matter of some doubt, mainly because certain combinations of mutants, namely K626+K745 and K626+K85, formed heterocaryons erratically. It was noted that when conidia of these mutants were mixed, growth could either occur quickly or after a long lapse of time (up to 10-12 days) or not at all. When growth was established, these heterocaryons continued to grow

FIG. 3.—Diagrams to show the orders of *his-1* allele differences, as inferred from distribution of flanking genetic markers amongst prototrophs, superimposed on the map order deduced from prototroph frequencies. Each arrow marks the proximal one of the pair of alleles joined by a given line. I, II and III are the three criteria used (see pp. 238-9). Each arrow point represents one cross.

vigorously. Their heterocaryotic conidia germinated without delay and, in their turn, formed cultures which grew vigorously on minimal medium. Both of the original components could be recovered from the heterocaryons.

These combinations have been further studied by introducing additional nutritional mutants* into K626, K745 and K85 so that forced heterocaryons, cultured on minimal medium supplemented with histidine, could be prepared. In turn, these were tested for their ability to grow on minimal medium, with the finding that generally all such heterocaryons involving K626 + K85 or K626 + K745 were unable to grow on minimal medium. Hence, it must be concluded that these combinations do not complement.

It was found that the forced heterocaryons sometimes began to grow, much as did some of the mixed inoculations in the original experiments. Some of these cultures were analysed by plating samples of their conidia on minimal medium supplemented with histidine and the other compounds needed by the original constituents of the heterocaryon. The resulting colonies were analysed for their nutritional requirements. In this way it was found that the growth of the heterocaryons in the absence of histidine was due to reversion of K626 to prototrophy. Thus, conidia from a heterocaryon formed between K626, 36104 and K745, Y2198, 5531, found capable of growth on minimal medium, gave colonies of four kinds: (i) requires histidine + methionine, (ii) requires histidine + tryptophan + pantothenic acid, (iii) grows in minimal medium, (iv) requires methionine. The first two are the parental constituents, the third could be their heterocaryon but it is not, while the fourth is a parental constituent in which K626 has reverted. The heterocaryotic colonies which can grow on minimal medium always contain the K626 reversion as one component.

However, the performances of forced heterocaryons, as well as further repeated experiments, confirm the original finding that the combination of K624 + K626 does complement slightly. The heterocaryon attains, on minimal medium, about 25 per cent. of the normal amount of growth shown on supplemented medium or by the wild type on minimal medium. Hence the *his-1* mutants fall into six groups rather than the eight groups previously reported. The members of one of these groups, comprising nearly all of the *his-1* mutants, are not complementary with any *his-1* mutants. The complementation map is shown in fig. 4. The relation between the complementation groups now recognised and those listed in Catcheside (1960) is set out in table 7.

We are now in a position to compare the genetic and complementation maps. In fig. 2, it will be seen that all mutants belonging to any one complementation group are in an individual region of the genetic map, unmixed with mutants belonging to any other complementation

* The nutritional markers used in these experiments were *me-3*(36104), *try-3*(td1) and *try-4* (Y2198) accompanied by *pan-1*(5531).

group. The A group, of non-complementing mutants, is in a proximal section of the genetic map, amounting to about a fifth of the known length of the map. These are followed by a section, about a fifth of the map, containing the E mutants and finally by the F mutants in the distal half to three-fifths of the map. Only the C mutant, K₇₄₄, may be amongst the more distal of the A mutants. However, its actual position cannot be determined with any certainty on the available evidence.

FIG. 4.—Complementation map of *his-1*.

TABLE 7

Synonymy of complementation groups of his-1 mutants mapped genetically

Present group	Previous groups (Catcheside, 1960)	Mutants mapped genetically
A	A	K83, K ₂₄₅ , K ₅₆₆ , K ₆₁₇ , K ₆₂₀ , K ₆₂₇ , K ₆₄₉ , C ₈₄
B	B	
C	C	K ₇₄₄
D	D	
E	E, G	K ₉₀ , K ₇₄₂ , Y ₁₈₉ -M ₈₉
F	F, H	K ₈₅ , K ₁₄₁ , K ₆₁₆ , K ₆₂₅ , K ₆₅₁

4. DISCUSSION

The primary purpose of the study of recombination between *his-1* mutants was to construct a genetic map to show the relative positions of the allelic differences, with the intention of making a comparison with the complementation map. It turns out that the relationship, so far as it is established, is a very simple one with mutants belonging to the same complementation group being grouped together in a distinct segment of the genetic map. Beyond the demonstration of a very good correlation of the two maps, there is only one special property. The notable feature, compared with other loci, is that the

non-complementing mutants appear to be concentrated at one end, the proximal end, of the map. The more usual situation, encountered in *pan-2* (Case and Giles, 1958), *me-2* (Murray, 1960, 1963) and *his-5* (Smith, 1965), is for the non-complementing mutants to be spread all along the genetic map, mingled with the sites of difference of the complementing mutants. *His-1* may not be unique in its proximal localisation of the sites of non-complementing mutants, for *his-3* shows a similar distribution (Catcheside, 1964), though with some of the kinds of complementing mutant apparently mingling with the non-complementing ones.

The gene which affects the frequency of recombination between alleles is of unusual interest. Although the mechanism of recombination at the molecular level is a matter of fundamental importance, theories which take account of the molecular architecture of deoxyribonucleic acid and of the time of its replication with respect to the time of genetic recombination at meiosis are only of very recent proposal (Holliday, 1962, 1964; Whitehouse, 1963; Hastings and Whitehouse, 1964; Whitehouse and Hastings, 1964). There is no need at this juncture to explain the various theories beyond saying that they include, variously, separation over short distances of the two chains of a DNA molecule, the breakage of one or both of these chains so separated, the formation of segments of hybrid DNA, the excision of mispaired bases or of segments of a DNA chain, and the reconstruction of segments of the DNA chain using information in the other chain. Many of these events involve chemical reactions, either the dissolution of bonds in breakage and excision or the formation of bonds in processes of reconstruction. It is characteristic of organisms that chemical processes of these kinds are catalysed by enzymes and that each kind of enzyme is structurally specified by a gene and is subject to control, directly or indirectly, by other gene systems. It is in these terms that some attempt must be made to understand the possible ways in which the gene *rec* acts.

In the first place, it appears that *rec* is fully recessive to *rec*⁺, one dose of *rec*⁺ being as effective as are two doses. It is an axiom that dominance implies that the gene product is metabolically active. Hence, there are two general modes of action of *rec*⁺, namely either it determines the structure of an enzyme which catalyses one of the many different events which make up genetic recombination or else it determines the structure of a regulatory substance which either activates or represses one of these enzymes. In practice, it would probably be difficult to distinguish between production of an enzyme and activation of an enzyme. Hence, consideration may be restricted to comparison of *rec*⁺ having a metabolic activity as compared with it repressing a metabolic activity. Furthermore, discussion may assume that the effects of the gene are general, rather than restricted in action to alleles of *his-1*; this assumption is being subjected to test.

The effects of substitution of *rec* for *rec*⁺ appear to be primarily a

more than ten-fold increase in the formation of prototrophs by crosses between allelic genes. This is not accompanied by any corresponding increase in recombination between neighbouring non-allelic genes. It does appear to be accompanied by some increase in the degree of asymmetry in the distribution of flanking markers amongst the prototrophs which are selected, but this effect is by no means firmly established. The most likely activity controlled by *rec*⁺ may well be some kind of monitoring enzyme which corrects faults which appear in the DNA. If it were an enzyme postulated to induce breaks in the nucleotide strands to allow hybrid DNA to form, one would expect the high frequency to be dominant to low frequency. If it were an enzyme which excised a particular kind of mispaired base one would again expect high frequency to be dominant to low frequency. Moreover, there should be other associated effects, consequent upon the specificity of the enzyme for a particular aspect of a particular defect. The direction of the correction may in the normal be expected to go either way, but in the mutant there would be a strong bias in one of these ways, leading most likely to a change in the distribution of flanking markers amongst the prototrophs. Further, the effect should be limited to those alleles, or pairs of alleles, capable of generating the particular defect in hybrid DNA.

The most plausible suggestion that can be made is that the effect is on the breakage process. For hybrid DNA to be formed, a break must exist for at least a short period of time. If there were a variation in the efficiency of a repair process, particularly one repairing breakages, the less efficient process would permit the more frequent formation of hybrid DNA and so the possibility of occurrence of the other events which lead to allelic recombination. This might be a means whereby the frequency of allelic recombination could change quite markedly without the distribution of flanking markers amongst the prototrophs being necessarily altered relatively. However, it is difficult to see why there should, at the same time, be no strong effect on the recombination of non-allelic genes, unless allelic and non-allelic recombination occur by effectively distinct mechanisms.

A remarkable feature of the recombination data collected from various fungi is the great variability which exists not only between species, but also between genes in the same species and between the same pairs of alleles. In the main, interest has been concentrated on fine structure mapping and on the mechanism of "conversion". In these regards, the variability observed has seemed more of a nuisance than a possible source of information about mechanisms (Catcheside, Jessop and Smith, 1964). The variability appears to concern all aspects of the recombination. Referring only to the more striking data in the literature, variation is seen to be manifested in the following ways: (i) prototroph frequency, *e.g.* for the *pan-2* alleles 5 and 3 (Case and Giles, 1958), (ii) the proportion of prototrophs showing recombination of the flanking markers, *i.e.* variation in the fraction $R_1 + R_2/T$,

e.g. the *pan-2* alleles 5 and 3 and the *inos* alleles 37401 and 64001 (Giles, 1956), (iii) the ratio of R₁ and R₂ types of prototrophs, *e.g.* the *cys* alleles 80702 and 48401 (Stadler, 1959), the *am* alleles 2 and 3 (Pateman, 1960) and the *inos* alleles 37401 and 64001, (iv) the ratio of P₁ and P₂ types of prototrophs, *e.g.* the *inos* alleles previously mentioned. Indeed, in the case of the *inos* alleles 37401 and 64001, the variations shown by the distribution of the flanking marker combinations are correlated with the variation in prototroph frequency. Some of the variations in the statistical characteristics of prototrophs are accompanied by differences in frequencies of recombination between non-allelic genes, but not apparently in any definitely systematic fashion. Nothing is known of the genetics of the variations in recombination frequency shown by non-allelic genes, *e.g.* the second division segregation frequencies shown by *lys-5* (= *asco*) (Stadler, 1956).

In addition to the variations mentioned, which are detectable amongst the prototrophs screened from samples of individual ascospores, other sorts of variation are detectable in asci examined whole. Some alleles, it seems, show appreciable frequencies of post-meiotic segregation, while others do not. It has not so far been shown whether this is exclusively a property of the particular allele itself or whether there may also be genetic differences of more general effect.

It is clear that there is an ample field for the exploration of genetic and other variations which affect recombination. A full understanding of these appears to be essential to any complete description of the molecular processes which give rise to genetic recombination.

5. SUMMARY

A fine structure map of some of the allelic differences found in mutations of the *his-1* gene has been constructed. The data used comprise the frequencies of prototrophs produced by allelic crosses and the distribution of flanking markers amongst these prototrophs. The genetic map and the complementation map of *his-1* correlate well as regards the complementing mutants. A notable feature, compared with other loci, is that the non-complementing mutants appear to be concentrated at the proximal end of the genetic map. They are not mingled with the majority of the complementing mutants.

A gene, *recombination*, which affects the frequency of recombination between alleles, has been found. The frequencies of prototrophs formed in allelic crosses are caused by it to alter by a factor greater than ten. The low frequency allele, *rec*⁺, is dominant to the high frequency allele, *rec*. The gene affects non-allelic recombination to a very small extent if at all. The significance of this gene for an understanding of genetic recombination at the molecular level is discussed.

Acknowledgment.—The work was supported by a research grant awarded by the Department of Industrial and Scientific Research.

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