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Recombination within the *am* gene of *Neurospora crassa*

By J. R. S. FINCHAM[1]

John Innes Institute, Bayfordbury, Hertford, Herts.

With Appendix by J. A. PATEMAN

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1. INTRODUCTION

Mutants which map in the *am* gene of *Neurospora crassa* have various kinds of defect in the structure of NADP-linked glutamate dehydrogenase (Fincham, 1962; Fincham & Stadler, 1965; Pateman & Fincham, 1966). Crosses between different *am* mutants yield low frequencies of wild-type progeny (Pateman, 1958, 1960) as would be expected if the mutations are at different sites within the gene. Pateman has shown that almost any pair of *am* mutants of independent origin will yield some wild-type recombinants when crossed together, and he was able to arrange the mutant sites in a provisional linear order on the basis of the recombinant frequencies. His data are summarized in the Appendix to this paper. In the present work an attempt has been made to place the map of the *am* gene on a firmer basis by making the genetic backgrounds of the stocks used as uniform as possible, and by studying the relationship between the formation of am^+ recombinants and the recombination of flanking markers. The markers used have been *inositol*, distal, and *spray*, proximal to the *am* locus. The results support a linear arrangement of the *am* sites broadly in agreement with Pateman's map, and further provide a strong indication that recombinants within the gene arise predominantly through a polarized 'conversion' process rather than through conventional crossing-over.

2. MATERIALS AND METHODS

(i) Neurospora *strains*

The mutants *inositol* (mutant R233, obtained from S. R. Gross) and *spray* (obtained from R. W. Barratt) were each recovered in strains of *A* mating type following six successive generations of crossing to the standard wild-type 74 *A* (ST *A*). The *am* mutants, which were selected for this study because of their diverse effects on glutamate dehydrogenase, had various origins which have been detailed elsewhere (Fincham, 1958; Fincham & Stadler, 1965). All, originally in strains of *a* mating type, were put through six successive generations of crossing to ST *A*. In each case *a am* derivatives of the sixth cross were crossed to *A inos* and *A sp*

[1] Present address: Department of Genetics, The University, Leeds, 2.

strains which had themselves been derived from six generations of inbreeding with ST *A*. From these crosses *a am sp*, *A am sp*, *a inos am* and *A inos am* derivatives were isolated and were used in the subsequent analysis. In most cases the *A am sp* strains finally selected for use were crossed to the original *a am* strains; the absence of am^+ recombinants from such crosses gave assurance that no confusions had occurred during the back-crossing programme (cf. Table 1). It was also shown that the finally derived strains retained the complementation characteristics and enzymic properties of the original mutants.

(ii) *Technique for crosses between* am *mutants*

In each cross a *sp* am^x strain was inoculated on to ten Petri dishes each containing 20 ml. of standard crossing medium (Westergaard & Mitchell, 1947) supplemented with 0·005 M monosodium L-glutamate. After 6 to 8 days of incubation at 25°C., about 1–2 ml. of a dense suspension of conidia of an *inos* am^y strain of opposite mating type was distributed over the surface of each culture. The plates were incubated for a further 1½–2 weeks at 25°C. in an inverted position. At the end of this time a large number of ascospores had usually been discharged on to the lid of each dish. The advantage of using the *sp* strain as the female parent in each cross lay in the fact that the *sp* mutation causes a great reduction in the production of conidia which are often an embarrassment in plate cultures.

Crosses were made between alleles in all combinations except those involving strongly complementary alleles which would have complicated the analysis because of the formation of pseudo-wild ascospores. Each inter-allele cross was made in two ways: *sp* am^x $inos^+ \times sp^+$ am^y *inos*, and sp^+ am^x *inos* × *sp* am^y $inos^+$. Such pairs of crosses will be referred to as reciprocal crosses.

(iii) *Plating of ascospores*

In the region of 6,000,000 ascospores were screened from most of the crosses. When a cross was highly fertile this number could easily be obtained from four of the crossing plates, but in the case of a few less-fertile crosses anything up to ten dishes had to be used. The ascospores were washed from the lid of each dish with 10 ml. of a sterile solution of 0·01% agar in water, the combined washings were filtered through a loosely packed filter of sterile cotton wool to remove fragments of mycelium, and the number of spores per millilitre was determined by haemacytometer count. The suspension was shaken vigorously prior to withdrawal of the sample for counting, and the viscosity due to the agar helped to hold the ascospores in suspension; even so it is thought that on occasion a representative sample was not obtained and that this probably accounts for the variation in results which was occasionally observed between repeated platings from the same cross (cf. Table 1). Between 400 and 500 ascospores were usually counted. The suspension was now held in a 60°C. water bath for 30 min. to induce germination, and appropriate volumes were pipetted into bottles containing molten minimal agar medium 'N'

(Vogel, 1956) with 1·5% sucrose as carbon source, 0·02 M glycine to inhibit growth of *am* sporelings (Fincham, 1950; Pateman, 1957), 10 μg./ml. inositol, and 5 μg./ml. achromycin (Lederle Laboratories). The antibiotic was added to prevent the growth of bacterial contaminants which otherwise proved troublesome on occasion. The resulting suspension was plated at the rate of about 15 ml., or about 10^5 spores, per dish.

(iv) *Detection of* am$^+$ *recombinants*

After 22–24 hours at 25°C., plates were scanned with a binocular dissecting microscope at a magnification of 12·5 ×. The *am*$^+$ recombinants were very clearly distinguishable against the background 'fuzz' of barely germinated *am* spores. The *am*$^+$*sp*$^+$ sporelings had produced long (up to 1 cm.) vigorous hyphae, often reaching the surface of the medium and branching near the tips, while *sp am*$^+$ sporelings formed compact and relatively dense spherical colonies.

This procedure was certainly more laborious than the more usual one of using sucrose-sorbose medium and scoring the resulting compact colonies by eye after 2 or more days' incubation. It was adopted for two reasons. Firstly, *sp* is much more easily distinguished from *sp*$^+$ on sucrose than on sucrose-sorbose medium. Secondly, with the high densities of plating which were necessary in order to obtain useful numbers of recombinants, it was feared that a more prolonged incubation might lead to heterokaryon formation and consequent mis-scoring of the flanking markers.

(v) *Testing recombinants for inositol requirement*

All the *am*$^+$ recombinants were transferred, sixteen to a plate, to the surface of minimal agar medium N containing 0·3% sucrose plus 1·0% sorbose to induce compact growth, and 0·02 M glycine to inhibit growth of *am* spores, but devoid of inositol. The *sp* colonies were transferred more or less intact, while in the case of *sp*$^+$ recombinants substantial hyphal branches were taken; in each case the transfers were made in blocks of agar of minimum size. After 2–3 days at 25°C., *inos*$^+$ recombinants had formed substantial colonies while *inos* recombinants had hardly grown out of the agar blocks in which they had been transferred.

3. RESULTS AND DISCUSSION

(i) *Validity of the method*

Table 1 shows the results of the analysis of the crosses. Two features of the data provide support for the assumptions on which further discussion will be based. First, *am*$^+$ spores are not produced in the control crosses in which the parents carried identical *am* alleles; hence *am*$^+$ spores must be due to recombination of some kind between different sites within the gene. Secondly, there is no overall tendency for a higher recovery of one allele rather than the other at either flanking locus; any

inequalities in one cross were generally balanced in the reciprocal cross. Hence the scoring of the markers seems to have been reliable and there are no important viability differences.

(ii) *Mapping by recombination frequencies*

Figure 1 shows an order of the mutant sites based on frequencies of am^+ recombinants. While the order given provides one of the best fits to the data, several other

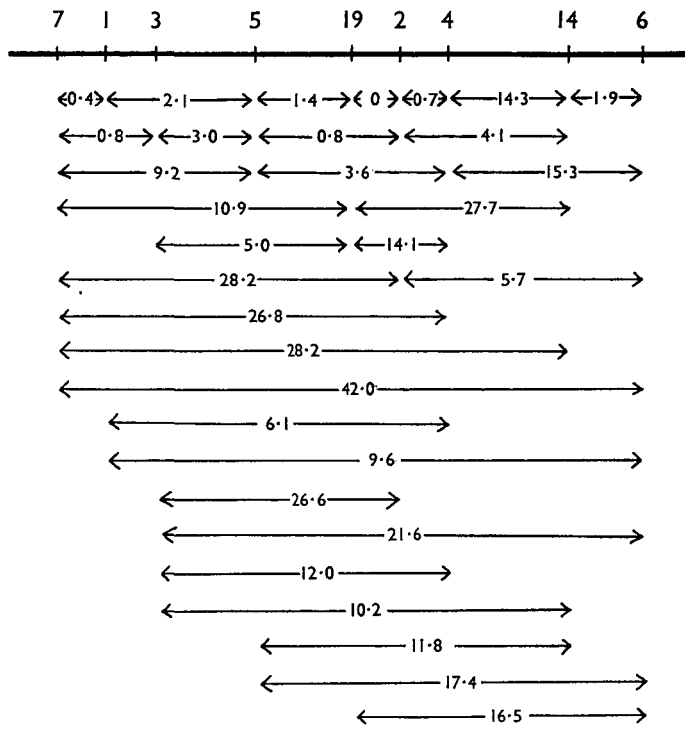

Fig. 1. Map of mutant sites based on frequencies of am^+ recombinants. The distances are given in terms of frequencies per 10^6 germinated spores, and are the means of the values from reciprocal crosses.

slightly different orders would be about as good. In particular the sequence of the very closely linked sites 1, 3 and 7 is not uniquely determined by the data. It will be seen that additivity of frequencies along the map is no more than approximate at best, and that there are some striking anomalies. There is a general tendency for map intervals to be more than additive ('map expansion', cf. Holliday, 1964). Thus the cross 6×7 gave an am^+ frequency of 40–50, while the sum of the intervals connecting 6 and 7 is only 19·6. The most striking individual deviation from additivity is shown by the sites 19, 2 and 4; 2×4 gave a frequency of recombinants of only $0{\cdot}7 \times 10^{-6}$ while 19×2 gave none in 10^7 spores, yet 19×4 gave a frequency of 14×10^{-6}. Among the other aberrations, 2×14, 1×4, 1×6 and 2×6 gave unexpectedly low frequencies in relation to the whole map while 19×14 and 3×2 gave

Table 1. *Results of crosses of the type* am^{x} sp × am^{y} inos

Cross	No. am^+ spores	Frequency of am^+ (per 10^6 germinated spores)	Genotypes with respect to flanking markers				% *sp-inos* recombination among am^+
			+ inos	*sp +*	*+ +*	*sp inos*	
$am^7 spA \times am^7 a$	0	0	0	0	0	0	—
$am^7 spa \times am^1 inosA$	3	0·4	1	1	1	0	33
$am^7 spa \times am^3 inosA$	5	1·1	2	1	1	1	40
$am^3 spA \times am^7 inosa$	4	0·6	1	3	0	0	0
$am^7 spa \times am^5 inosA$	74	10·9	23	13	18	20	51
$am^5 spA \times am^7 inosa$	49	7·4	12	17	5	**15**	41
$am^7 spa \times am^{19} inosA$	197	15·2	50	45	**66**	36	52
$am^{19} spA \times am^7 inosa$	34	6·6	5	**14**	10	5	44
$am^7 spa \times am^2 inosA$	271	22·2	**109**	36	**76**	50	47
$am^2 spA \times am^7 inosa$	178	34·2	31	40	23	**84**	60
$am^7 spa \times am^4 inosA$	133	26·6	47	33	27	26	40
$am^4 spA \times am^7 inosa$	136	27·0	23	**61**	25	27	38
$am^7 spa \times am^{14} inosA$	74	15·2	**34**	10	18	12	40
$am^{14} spA \times am^7 inosa$	221	41·2	54	72	35	**60**	43
$am^7 spA \times am^6 inosa$	42	37·5	12	15	2	**13**	36
———————*	153	34·0	57	51	27	18	29
———————*	176	40·4	57	52	26	41	38
$am^6 spA \times am^7 inosa$	164	51·2	**83**	29	30	22	32
———————‡	93	30·0	30	31	19	13	34
———————‡	312	52·8	**110**	73	53	**76**	41
$am^1 spa \times am^5 inosA$	17	2·6	7	4	2	4	35
$am^5 spa \times am^1 inosA$	12	1·6	3	3	3	3	50
$am^1 spA \times am^4 inosa$	59	7·8		**46** + : 13 *sp*†			—
$am^4 spa \times am^1 inosA$	26	4·4	6	10	2	8	38
$am^1 spA \times am^6 inosa$	22	10·8	**13**	1	5	3	38
$am^6 spa \times am^1 inosA$	136	8·5	14	**84**	25	**43**	50
$am^3 spA \times am^3 a$	0	0	0	0	0	0	—
$am^3 spa \times am^5 inosA$	10	1·4	4	3	2	1	30
$am^5 spa \times am^3 inosA$	32	4·5	11	8	9	4	41
$am^3 spa \times am^{19} inosA$	44	5·2	15	11	13	5	41
$am^{19} spa \times am^3 inosA$	35	4·7	7	**16**	3	9	34
$am^3 spA \times am^2 inosa$	210	28·0	**96**	33	40	41	39
$am^2 spA \times am^3 inosa$	110	25·3	28	**49**	21	12	30
$am^3 spA \times am^4 inosa$	75	13·2	**33**	12	**22**	8	40
$am^4 spa \times am^3 inosA$	61	10·9	15	21	6	**19**	41
$am^3 spA \times am^{14} inosa$	52	8·8	**26**	2	13	11	46
$am^{14} spa \times am^3 inosA$	84	11·5	8	**31**	10	**35**	53
$am^3 spA \times am^6 inosa$	104	20·6	**51**	10	**28**	15	41
$am^6 spa \times am^3 inosA$	115	22·5	15	**48**	19	**33**	44
$am^5 spA \times am^5 a$	0	0	0	0	0	0	—
$am^5 spa \times am^{19} inosA$	9	1·1	5	1	1	2	33
$am^{19} spa \times am^5 inosA$	2	1·8	1	1	0	0	0
$am^5 spA \times am^2 inosa$	13	1·0	6	2	0	5	38
$am^2 spa \times am^5 inosA$	2	0·6	0	1	1	0	50
$am^5 spA \times am^4 inosa$	11	3·9	**7**	0	3	1	36

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Table 1—*continued*

Cross	No. am^+ spores	Frequency of am^+ (per 10^6 germinated spores)	Genotypes with respect to flanking markers				% *sp-ions* recombination among am^+
			+ inos	*sp +*	*+ +*	*sp inos*	
$am^4spa \times am^5inosA$	14	3·3	1	**10**	1	2	21
$am^5spA \times am^{14}inosa$	145	13·0	**80**	20	14	**31**	31
$am^{14}spa \times am^5inosA$	66	10·6	6	**36**	9	15	36
$am^5spA \times am^6inosa$	124	18·8	**70**	13	18	23	33
$am^6spa \times am^5inosA$	107	16·0	13	**50**	23	21	41
$am^{19}spa \times am^2inosA$	0	0	0	0	0	0	—
$am^2spa \times am^{19}inosA$	0	0	0	0	0	0	—
$am^{19}spA \times am^4inosa$	28	13·3	11	8	4	5	32
$am^4spa \times am^{19}inosA$	89	14·9	23	34	22	10	35
$am^{19}spa \times am^{14}inosA$	48	28·2	**23**	12	3	10	27
$am^{14}spa \times am^{19}inosA$	127	27·2	23	**57**	28	19	37
$am^{19}spA \times am^6inosa$	53	12·0	**32**	6	11	4	28
$am^6spa \times am^{19}inosA$	137	21·1	26	**54**	28	29	42
$am^2spA \times am^2a$	0	0	0	0	0	0	—
$am^2spA \times am^4inosa$	2	0·4	2	0	0	0	0
$am^4spa \times am^2inosA$	8	1·0	2	1	1	4	62
$am^2spA \times am^{14}inosa$	25	4·6	**12**	3	7	3	40
$am^{14}spa \times am^2inosA$	18	3·6	4	10	2	2	22
$am^2spA \times am^6inosa$	50	5·5	**26**	10	7	7	28
$am^6spa \times am^2inosA$	37	5·9	8	16	6	7	35
$am^4spa \times am^{14}inosA$	102	17·3	**48**	16	17	21	37
$am^{14}spA \times am^4inosa$	60	11·3	11	**27**	8	14	37
$am^4spA \times am^6inosa$	84	18·4	26	22	23	13	43
$am^6spa \times am^4inosA$	69	12·3	15	24	13	17	43
$am^{14}spA \times am^{14}a$	0	0	0	0	0	0	—
$am^{14}spA \times am^6inosa$	12	1·4	7	3	1	1	17
$am^6spa \times am^{14}inosA$	16	2·3	6	8	1	1	12
$am^6spA \times am^6a$	0	0	0	0	0	0	—

Numbers in significant ($P < 0·05$) excess over reciprocal class shown in heavy type.
* Repeated crosses.
‡ Repeated platings of a repeated cross.
† Not scored for *inos*.

unexpectedly high ones. The best that can be said for Fig. 1 is that the inconsistencies shown are perhaps no worse than have been quite frequently encountered in attempts to map within *Neurospora* genes by crude recombination frequencies.

(iii) *Mapping by reference to flanking markers*

The data shown in Table 2 lead to estimates of the *sp-am* and *am-inos* distances as 6·0 and 8·8 map units respectively. If the *am* gene is colinear with the chromosome, and if all the am^+ recombinants were generated by orthodox crossing-over,

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one would expect that, with respect to the flanking markers, one recombinant genotype, R1, would be in the great majority, the two parental genotypes, P1 and P2, much less frequent, and the other recombinant type, R2, rare among the am^+ recombinants (Pritchard, 1955; Case & Giles, 1958). Given the values for the *sp-am* and *am-inos* intervals, and assuming no interference, we would expect the frequencies P1 = 8·3, P2 = 5·5, R1 = 85·7 and R2 = 0·5. The constitutions of P1 and P2, and of R1 and R2, would, of course, be reversed in reciprocal crosses. If, on the other hand, am^+ recombinants were generated by some process which was quite uncorrelated with crossing-over we would expect P1 = 43·2, P2 = 43·2, R1 = 6·8,

Table 2. *Linkage of* am *to flanking markers*
Cross: *spA* × am*^{3} *inosa*, i.e. *sp* + + × + *am inos*
Ascospores were germinated and isolated at random; germination was *ca.* 95%

Genotype	Number of ascospores
sp + +	220
+ *am inos*	211
sp am inos	13
+ + +	14
sp + *inos*	18
+ *am* +	23
sp am +	3
+ + *inos*	0
Total	502

Map distances:
sp-am 6·0
am-inos 8·8

* 6 × backcrossed to ST *A*.

R2 = 6·8. Examination of the data of Table 1 shows that neither situation holds in the present case. A number of generalizations can, nevertheless, be made:

(1) There is clearly a correlation between the formation of am^+ recombinants and recombination between *sp* and *am*, since, among am^+ ascospores, the *sp-inos* recombination frequency is almost invariably much higher than the 13·8% expected if there were no correlation.

(2) In the great majority of crosses the *majority* of the am^+ recombinants are still non-recombinant with respect to *sp* and *inos*, the *sp-inos* recombination frequency being usually in the range 30–50%. Thus, while a correlation with crossing-over does exist, the generation of am^+ recombinants, far from being dependent on crossing-over, does not even entail a 50% chance of it.

(3) The 'classical' pattern R1 ⩾ P1 ~ P2 ⩾ R2 is nowhere approached. In a number of pairs of reciprocal crosses (e.g. 7 × 2, 7 × 14, 1 × 6, 3 × 4) there is an approximation to the pattern P1 = R1 > P2 = R2, which was a conspicuous feature of the data of Murray (1963) on *me*-2, Stadler & Towe (1963) on *cys* and Smith (1965) on *his*-5, all in *Neurospora*. More numerous, however, are cases which approximate

to P1 > P2 = R1 = R2; a similar pattern was found in a few of Smith's crosses and several of Murray's. In addition there were a number of crosses in which there were no significant deviations from equality among the four classes; these were mostly crosses from which the numbers of am^+ recombinants were too small for the detection of anything but a gross inequality. Where significant inequalities existed they were usually reversed in the reciprocal cross, so that, taking pairs of reciprocal crosses together, P1 ~ P2 and R1 ~ R2.

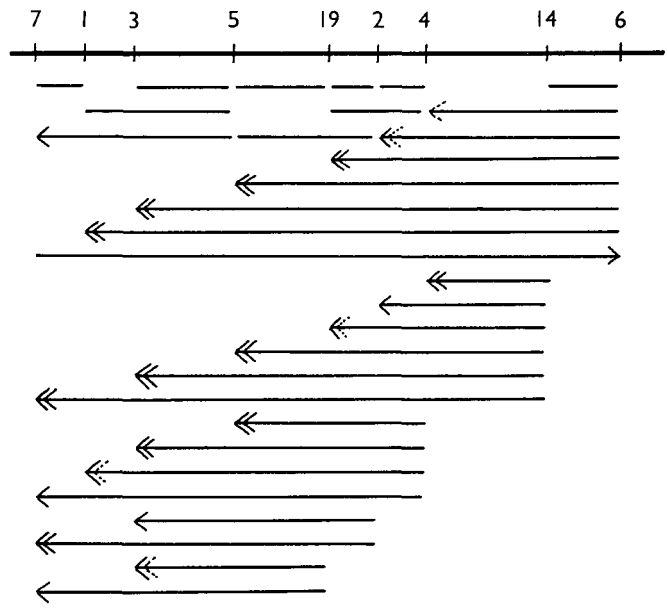

Fig. 2. Map of mutant sites based on the distribution of the *sp* marker. Arrows pointing right to left indicate linkage of the presumed proximal wild-type site to *sp*; $P < 0{\cdot}05$, or 0·05–0·2 in the case of dotted arrow heads. Arrows pointing left to right indicate 'reverse' linkage to *sp*. Double arrows: results from reciprocal crosses. single arrows: result from one cross only. Lines without arrowheads: no asymmetry in distribution of the *sp* marker ($P > 0{\cdot}2$).

Taking the crosses as a whole it is possible to arrange the *am* mutant sites in a linear order such that the presumptive proximal site in any pair shows linkage to the proximal marker *sp*. If we consider only the crosses approximating to the pattern P1 ~ R1 > P2 ~ R2 it is also true that the presumptive distal site shows no significant linkage to the *inos* marker. In those crosses showing the pattern P1 > P2 ~ R1 ~ R2, however, the distal site tends to show reverse linkage with *inos*. By far the most consistent order is that obtained by reference to the proximal marker *sp*. To apply Smith's (1964) criteria, criterion 2 (proximal linkage) leads to an almost completely consistent order, criterion 3 (distal linkage) gives conflicting results, while the classical criterion 1 (predominance of one of the two marker recombinant classes) is applicable in only a few cases (e.g. 2×7, 3×4, 7×19).

Figure 2 shows the ordering of the sites by reference to *sp*. The result is, so far as it goes, in agreement with Fig. 1, although the order within each of the three groups

of sites (1, 3, 7), (2, 4, 19) and (4, 6) is not defined. Within these groups a most probable (though not certain) order can be deduced from the recombination frequency data. The 'polarity map' shows a high degree of internal consistency with the exception of the cross 6 × 7 which shows no linkage, or even reverse linkage, of the proximal site 7 to *sp*.

(iv) *Possible mechanisms of recombination within* am

It seems simplest to interpret these data as meaning that am^+ recombinants are generated by a gene conversion process which is correlated with crossing-over but not dependent upon it. The excess of one parental marker combination can be taken as indicating that the *am* mutant site originally linked with this combination is converted to wild type more often than the *am* mutant site contributed by the other parent. Viewed in this way the data can be taken as indicating that there is a gradient of conversion frequency along the *am* gene, distal sites (i.e. on the *inos* side) being converted preferentially as compared with proximal ones, which thus tend to remain in their original linkage with *sp*. Murray made a similar interpretation of her data on the *me*-2 gene. This type of explanation is essentially the same as the *polaron* hypothesis of Lissouba *et al.* (1962). The existence in rather high frequencies of am^+ recombinants of the minority parental marker class and of the two recombinant classes can be explained in a general way by saying (*a*) that the polarity is only relative and that both mutant sites in a cross can be converted to wild type though often with unequal frequencies, and (*b*) that conversion is correlated with crossing-over. To explain the rather frequent occurrence in these data, and the very general occurrence in the data of Murray (1963), Smith (1965) and Stadler & Towe (1963), of patterns of the type $P1 \sim R1 > P2 \sim R2$ it is sufficient to say that correlated crossing-over tends to occur preferentially on the side of the gene (in this case the distal side) that shows preferential conversion.

The data could also be interpreted within the framework of either of the 'hybrid DNA' hypotheses elaborated respectively by Holliday (1964) and Whitehouse & Hastings (1964). In either case one could say (*a*) that, within the *am* region, hybridization of the DNA of homologous chromatids tends to spread into the gene from the *inos* side, (*b*) that conversion at one site and not at the other (which is necessary if an am^+ strand is to be formed) can occur most easily if the region of hybridity includes the first site and not the second, and (*c*) that crossing over tends to occur (though with less than 50% frequency) in the hybrid region. The third point must be qualified to the extent that, in order to account for the general appearance of substantial numbers in *both* outside marker recombinant classes, one has to suppose that recombination within the gene is often accompanied by nearby crossing-over outside the hybrid region, perhaps in a different hybrid region. Such a clustering of recombinational events would be consistent with the concept of localized regions of close pairing between synapsed chromosomes (Pritchard, 1960).

The major inconsistency in the data—the non-polarity or even reversed polarity in the cross 6 × 7—is difficult to explain. One might suppose that hybrid DNA can spread into the gene to a limited extent from the proximal side and that this results in the most proximal site (i.e. 7) being converted as often through being in proximal hybrid DNA as is site 6 through being in distal hybrid DNA. The difficulty then is to explain why it is only in crosses with 6 that this high conversion frequency of 7 shows up. A formally adequate explanation would be that when a hybrid DNA region of proximal origin crosses site 7 it tends, for some unknown reason, to include all other sites up to but not including 6, so that only in 7 × 6 crosses would the hybrid region end between the two mutant sites. Such an explanation seems too special to carry much conviction. It may be, rather, that certain crosses show atypical patterns of hybrid DNA formation because of effects of the mutant sites themselves.

Strong effects of genetic background on recombination frequency are indicated by the comparison with the earlier data of Pateman, appended to this paper.

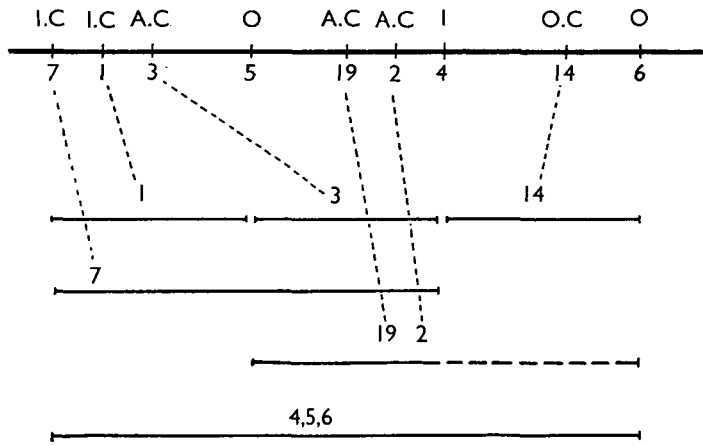

Fig. 3. Genetic map compared with the complementation map. Symbols: 0, mutant produces no detectable gene product; A, mutant produces potentially active glutamate dehydrogenase; I, mutant produces protein resembling glutamate dehydrogenase but always inactive; C, complementing mutant. The broken line indicates that am^2 and am^{19} complement very poorly with am^{14}.

Though the order of sites derived by Pateman is very much the same as suggested here, and the recombination frequencies are in general of the same order of magnitude, certain crosses show widely different frequencies in the two studies.

(v) *Relation of genetic map to properties of mutants*

There is no evident relationship between the map positions of the mutations within *am* and the type of effect on the protein product, i.e. glutamate dehydrogenase. Two mutants producing no detectable mutant glutamate dehydrogenase

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protein, 5 and 6, map relatively far apart, and from Pateman's data, 6 and 11, which also comes within this category (Roberts & Pateman, 1964), span the entire known gene map. Mutants producing recognizable abnormal varieties of the enzyme which all, with the exception of 4, show complementation in some combinations (Fincham & Stadler, 1965) are scattered throughout the central and proximal regions of the map. The anomalous mutant 14, which complements several other *am* mutants and yet produces no recognizable mutant protein, maps near the distal end. There is some apparent relationship between the recombinational and complementation maps (Fig. 3) but no more than could be due to chance.

SUMMARY

Maps have been made showing the order of *am* mutant sites using (*a*) frequencies of am^+ recombinants from crosses between *am* mutants and (*b*) the modes of distribution of the am^+ recombinants among the two parental and two recombinant classes with respect to the flanking markers *inos* and *sp*.

It is possible to arrange the sites in an order such that, in almost all the crosses yielding useful numbers of am^+ recombinants, the *sp* allele originally in coupling with the distal *am* mutant site occurs in the majority of the recombinants. No such consistent pattern was found with respect to the *inos* marker. The order obtained by reference to *sp* agreed with the best order deducible from recombination frequencies.

The data are consistent with the hypothesis that am^+ recombinants arise by a process of gene conversion, that there is a gradient of conversion frequencies from the right (*inos* or distal) end of the gene to the left (*sp* or proximal) end, and that conversion tends to be associated (though less than 50% of the time) with crossing-over, especially on the distal side.

There is no obvious relationship between the map position of a given *am* mutant and the properties of the protein product of the mutant gene.

I wish to thank Mrs Carole Fenning and Mrs Josephine Slarke for their assistance. Mrs Fenning carried out all the preliminary inbreeding. She and Mrs Slarke, during different periods of the work, set up the crosses, made the spore counts, and plated the spores.

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APPENDIX: An earlier study without the use of flanking markers

BY J. A. PATEMAN

Department of Genetics, University of Cambridge

Some years previous to the work reported in the main part of this paper, crosses were made between all pairs of *am* mutants then available except strongly complementary pairs. The strains used were not closely inbred but all had the standard wild-type 74 *A* in their immediate ancestry. Origins of the mutants are given in Fincham (1959). The methods for plating ascospores and scoring recombinants were essentially as described in the main paper. The results are summarized in Table 1.

As a means of reducing the data to order a series of linear diagrams were constructed, each one representing the results of one mutant crossed to all the others. In each diagram each site was placed either to the left or to the right of the common site at a distance from it proportional to the prototroph frequency given by the corresponding cross. The choice between left and right in each case was made so as

to make the different diagrams consistent as to order so far as possible. A good measure of general agreement between diagrams was achieved, with conflicts confined to the relative positions of closely linked sites. The best order appeared to be:

11—9—1—3—7—5—8—2—4—6

Furthermore, a statistical analysis (undertaken with the co-operation of Dr A. R. G. Owen) followed by enumeration on a computer gave the same order as the most probable one taking the data as a whole. The actual numerical values for

Table 1. *Frequencies of wild types from crosses between* am *mutants*

	Wild types			Wild types	
Cross	No.	Frequency per 10^6 plated ascospores	Cross	No.	Frequency per 10^6 plated ascospores
1 × 1	2	0·9 (?)	4 × 4	0	0
1 × 4	54	27	4 × 5	16	5
1 × 5	30	24	4 × 6	44	15
1 × 6	76	25	4 × 7	91	23
1 × 7	12	4	4 × 8	226	34
1 × 8	34	28	4 × 9	202	67
1 × 9	6	4	4 × 11	320	74
1 × 11	7	3			
			5 × 5	0	0
2 × 2	0	0	5 × 6	120	20
2 × 4	4	1	5 × 7	67	13
2 × 5	6	2	5 × 8	22	7
2 × 6	19	8	5 × 9	70	22
2 × 7	88	16	5 × 11	81	20
2 × 8	2	1			
2 × 9	192	35	6 × 6	0	0
2 × 11	91	26	6 × 7	231	46
			6 × 8	74	30
3 × 3	0	0	6 × 9	75	38
3 × 4	70	16	6 × 11	555	82
3 × 5	41	10			
3 × 6	92	15	7 × 7	0	0
3 × 7	3	1	7 × 8	54	21
3 × 8	142	19	7 × 9	12	3
3 × 9	7	1	7 × 11	51	6
3 × 11	8	1			
			8 × 9	57	31
			8 × 11	171	85
			9 × 11	9	4

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the map distances (in centimorgans $\times 10^4$) between adjacent sites, with their standard deviations, were as follows:

11—9	$4{\cdot}42 \pm 0{\cdot}41$	5—8	$3{\cdot}51 \pm 0{\cdot}77$
9—1	$2{\cdot}01 \pm 0{\cdot}54$	8—2	$2{\cdot}38 \pm 0{\cdot}66$
1—3	$1{\cdot}34 \pm 0{\cdot}51$	2—4	$12{\cdot}99 \pm 0{\cdot}79$
3—7	$1{\cdot}34 \pm 0{\cdot}51$	4—6	$7{\cdot}09 \pm 0{\cdot}62$
7—5	$16{\cdot}02 \pm 0{\cdot}72$		

With the exception of the sites 1, 3 and 7, which are too close together for their relative positions to be certain on either analysis, the order given is in good agreement with that derived in the main part of this paper.