A NEW MAP OF THE *amination-1* LOCUS OF *NEUROSPORA CRASSA*, AND THE EFFECT OF THE *recombination-3* GENE

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Abstract

The sites of difference from the normal gene of 13 *am-1* alleles have been mapped, almost without ambiguity, in *rec-3* background. Repulsion-phase crosses were made between pairs of the auxotrophic mutants and prototrophic recombinants selected. The recombinants were then classified into the four possible classes specified by different combinations of chromatids flanking *am-1*, by using markers at the closely linked loci *sp* and *his-1*. The sites of difference of the alleles were ranked in an order obtained using two criteria—the first based on significant differences between the two classes of prototrophic recombinants with non-parental combinations of flanking regions, and the second based on prototrophs with parental combinations. The unambiguous order, proximal to distal, of 11 sites is 6–14–4–2–5–17–1–7–11–3–9. The site of 19 is very close to 2, immediately adjacent either proximally or distally. The site of 8 could not be related unambiguously to the sites of 2 and 19, but it is placed unambiguously between 4 and 5. The data suggest that recombination in *am-1* frequently results from conversion without associated crossing-over, and that a gradient in the frequency of such conversion occurs, with more always occurring proximally.

Next, *rec-3*$^+$ crosses were made between *am-1* allele 6 and the other 12 alleles. Prototroph frequencies were reduced 10- to 36-fold compared with *rec-3* crosses. For most alleles, the *rec-3*$^+$ gene also significantly reduced the asymmetry between the two classes of prototrophs with parental combinations of flankers. Thus the dominant *rec-3*$^+$ gene reduces recombination throughout *am-1*, but seems to have a stronger effect in the proximal region.

If the genetic map obtained is folded once, it can be correlated with a linear complementation map of the 13 alleles. The parts of the gene responsible for various functions in the active *am-1* gene product are identified.

I. INTRODUCTION

An unambiguous map of the *amination-1* (*am-1*) locus of *Neurospora crassa* would be useful for several reasons. First, the genetic sites of mutants could be correlated with the sites of their amino acid alterations in the gene product, NADP-linked glutamate dehydrogenase (Wootton *et al.* 1973). Together, these should help us understand more about the action of the enzyme and the complementation between certain pairs of *am-1* mutants (Fincham and Stadler 1965). Second, the effect of the *recombination-3* (*rec-3*) gene (Catcheside 1966*a*) on the extent and pattern of recombination in *am-1* could be more accurately assessed. So far recombination

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between five alleles has been tested, with the dominant *rec-3*$^+$ gene reducing the frequency in every cross, the reduction ranging from 6- to 26-fold (Catcheside 1968). Using two alleles, Smyth (1971) has shown that there is apparently more reduction in the proximal region. These findings need to be broadened by using more alleles whose sites have been accurately mapped.

Fincham (1967) has attempted to map nine sites within *am-1*. He made pairwise crosses between auxotrophic *am-1* mutants and selected prototrophic recombinants. The regions flanking either side of *am-1* were marked with genetic differences at closely linked loci. The prototroph frequencies he obtained were low, indicating that he was using *rec-3*$^+$ stocks (Catcheside 1968), and they did not allow the sites of the mutants to be ordered uniquely in one dimension. The order he gave is one of several with as few, but still significant, ambiguities. Mapping by prototroph frequency rarely gives an unambiguous map (see Whitehouse and Hastings 1965). Fincham also ordered the alleles by considering the distribution of proximal flanking markers among prototrophs. He placed the allele which entered the cross with the rarer proximal flanker more proximal within *am-1*. This arbitrary criterion gave an order in reasonable agreement with one from prototroph frequencies, but ambiguities remained, especially between mutant pairs yielding few prototrophs.

In this report, an attempt was made to obtain an unambiguous map of 13 sites in *am-1*. Crosses similar to Fincham's (1967) were made, but *rec-3* stocks were used to obtain higher yields. Eleven of the sites were ordered without ambiguity. Subsequently, all alleles were tested in *rec-3*$^+$ crosses so that the *rec-3* effect could be gauged across the whole *am-1* locus.

II. Methods

Thirteen *am-1* alleles were obtained from various sources listed below:

Allele	Mutant No.	Source*	Allele	Mutant No.	Source*
1	32213	DGC83	8	—	FGSC788
2	47305	DGC3492	9	—	FGSC789
3	S2929	FGSC1186†	11	—	FGSC790
4	1381	DGC3491†	14	—	FGSC1190
5	B501	DGC7237	17	—	DGC12454
6	K314	DGC1005	19	—	FGSC1191
7	K410	DGC2035			

* DGC, D. G. Catcheside's Stock No.; FGSC, Fungal Genetics Stock Center No., California State University Humboldt Foundation, Arcata, California 95521.

† These two stocks are *rec-3*$^+$. All others are *rec-3*.

All these alleles are auxotrophic mutants (Fincham and Stadler 1965), requiring a source of α-amino nitrogen for growth. The *colonial temperature-sensitive-1* (*cot-1*) mutant C102t was incorporated for ease of handling, and *rec-3* constitutions determined by pedigree analysis or by crossing to *am-1*^{6} testers (Catcheside 1966*a*). The flanking markers *spray* (*sp*, B132), a morphological mutant mapping 5 units proximal to *am-1*, and *histidine-1* (*his-1*, K83), 3 units distal, were also incorporated, one or the other, into *am-1* mutant stocks (map distances are from Smyth 1973). These mutants have no effect on viability (Smyth 1971). In this paper *sp* and *his-1* will be used to symbolize the mutant allele at these loci; the wild-type allele will be symbolized +.

Control crosses were made between stocks carrying the same *am-1* mutant to determine the frequency of prototrophs attributable to reversion. The highest frequency was from *am-1*^{17} intercrosses, where two prototrophs occurred among 13·4 million viable offspring. The limit of 95%

confidence below which reversion frequency lies in this case is 0·054 per 10^5 offspring; for other allelic crosses it was lower.

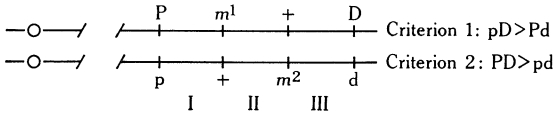
Experimental methods have already been described (Catcheside 1966*a*; Smyth 1971).

III. Map of *am-1*

(*a*) *Criteria of Mapping*

Crosses of the type *sp am-1*^{x} + × + *am-1*^{y} *his-1* were made in *rec-3* background and prototrophic *am-1*$^+$ progeny selected and scored for the *sp* versus *sp*$^+$ and *his* versus *his*$^+$ phenotypes. The frequency of prototrophs was not used for mapping because of previous failures. Instead, two criteria were based on the representation of flanking markers among prototrophic progeny.

Criterion 1 (the Classical Criterion) is based only on those prototrophs with recombinant associations of flanking genes. It states that the *am-1* allele with the more proximal site is that which entered the cross with the proximal flanking marker represented less frequently among such progeny and with the distal flanker represented more frequently. *Criterion 2* (the Polarity Criterion) is restricted to the other two classes, those with parental associations of markers. The more proximal *am-1* allele is defined as that which entered the cross with the same association of flankers as the more frequent of these two classes. These criteria can be related to the following diagram (Catcheside 1966*b*):



where m^1 and m^2 represent the sites of any two *am-1* mutants; P, p and D, d are general terms for markers at the proximal *sp* and distal *his-1* loci respectively; and haploid + + progeny are selected. The site of m^1 is placed proximal to that of m^2 since the class represented pD is then greater than Pd (Criterion 1) and PD larger than pd (Criterion 2).

Criterion 1 is based on the expectation that p+ +D recombinants will be more frequent than P+ +d progeny, since the former may arise either from a cross-over in region II alone, or a conversion of m^1 or m^2 to + with an associated cross-over terminating in regions *neighbouring* the conversion site, whereas the latter class (P+ +d) require at least one conversion of $m \rightarrow +$ with an associated cross-over, one terminus of which is in a *non-neighbouring* region. Criterion 1 is absolute, and orders the sites with respect to the centromere. In contrast, Criterion 2 is arbitrary and implies that conversion without associated crossing-over is polarized in the *am-1* locus in a uniform, proximal to distal, direction. That is, $m^1 \rightarrow +$ (yielding P+ +D prototrophs) is always more frequent than $m^2 \rightarrow +$ (p+ +d) wherever the sites are within *am-1*. This is so for the sites of *am-1*^{6} and *am-1*^{11} (Smyth 1971), but the use of this criterion depends on further data showing that this proximal excess is consistent throughout *am-1*.

In all crosses, sites were ordered by these two criteria only if differences were significant at the 5% probability level.

(*b*) *Results*

Thirteen *sp am-1*^{x} stocks (or their inbred offspring) were intercrossed with 13 *am-1*^{y} *his-1* lines, all stocks being *rec-3*. The prototrophic *am-1*$^{+}$ progeny were

TABLE 1

FREQUENCIES AND DISTRIBUTIONS OF FLANKING MARKERS OF *am-1*$^{+}$ PROGENY FROM *rec-3* CROSSES OF THE GENERAL CONSTITUTION *sp am-1*^{x} + × + *am-1*^{y} *his-1*

Parents		Frequency of *am-1*$^{+}$ progeny per 10^5 spores	S.E.	Distribution of flanking markers of *am-1*$^{+}$ progeny				
am-1^{x} *sp* +	*am-1*^{y} + *his-1*			*sp* +	+ *his*	+ +	*sp his*	Total
14	6	13	0·77	33	138	34	35	240
4	6	60	2·3	25	159	29	27	240
2	6	34	1·5	35	137	34	34	240
19	6	73	3·4	31	209	45	75	360
8	6	57	2·1	48	399	82	111	640
5	6	63	4·1	19	158	30	33	240
17	6	130	6·8	13	164	30	33	240
1	6	48	2·7	23	144	35	38	240
7	6	98	4·7	25	159	31	25	240
11	6	85	5·9	22	140	37	41	240
3	6	72	2·5	43	383	107	107	640
9	6	76	4·3	10	149	34	47	240
6	11	62	3·5	161	19	40	20	240
14	11	80	3·6	149	46	31	14	240
4	11	49	2·9	141	23	42	34	240
2	11	51	3·5	131	36	35	38	240
19	11	34	2·3	99	68	30	43	240
8	11	33	1·6	187	220	92	101	600
5	11	9·5	0·65	116	51	28	17	212
17	11	6·1	0·39	119	49	39	33	240
1	11	2·0	0·12	113	67	49	57	286
7	11	7·9	0·28	253	205	138	164	760
3	11	0·44	0·035	23	75	22	31	151
9	11	2·8	0·17	61	161	34	28	284
7	8	45	1·6	56	97	32	55	240
3	8	30	2·5	11	76	18	15	120
8	9	19	1·8	88	17	10	5	120
7	3	2·9	0·14	176	105	72	83	436
7	9	9·7	0·72	47	20	20	33	120
3	9	0·53	0·038	90	52	24	24	190
2	19	0·027	—	1	0	1	0	2
2	14	18	1·4	13	83	13	11	120
2	4	3·9	0·28	43	126	21	19	209
2	8	7·9	0·32	112	196	57	35	400
8	2	8·1	0·47	95	38	21	46	200
2	5	5·0	0·40	71	19	15	15	120
2	17	38	2·5	84	6	13	17	120
7	2	48	3·7	39	180	41	54	314

TABLE 1 (*Continued*)

Parents		Frequency of	S.E.	Distribution of flanking markers of *am-1*$^+$ progeny				
am-1^x *sp* +	*am-1*^{y} + *his-1*	*am-1*$^+$ progeny per 10^5 spores		*sp* +	+ *his*	+ +	*sp his*	Total
14	19	66	2·9	84	11	12	13	120
4	19	9·2	0·63	75	15	14	16	120
8	19	5·9	0·32	133	180	30	52	395
19	8	6·7	0·50	66	74	43	22	205
5	19	3·3	0·30	16	79	9	16	120
17	19	24	1·4	23	57	24	16	120
7	19	36	2·4	23	59	19	19	120
4	14	19	1·1	11	75	10	24	120
8	14	37	1·7	19	72	14	15	120
4	8	16	0·98	58	17	29	16	120
5	8	7·5	0·63	28	66	5	21	120
8	17	39	2·4	59	31	16	14	120
5	17	9·2	0·80	76	24	10	10	120
5	7	11	1·1	62	19	23	16	120
7	17	6·8	0·62	25	63	13	19	120
1	4	23	1·2	12	74	11	23	120
1	8	29	1·5	9	66	21	24	120
1	5	14	0·90	20	55	19	26	120
1	17	1·1	0·09	31	60	28	21	140
1	7	3·2	0·81	10	1	2	4	17
7	1	4·6	0·40	37	52	22	29	140

assayed for frequency and at least 120 prototrophs from each cross were then tested for flanking marker composition. Six of the 78 possible crosses involving different pairs of the 13 alleles were impracticable because of strong complementation (Fincham and Stadler 1965). 55 of the remaining 72 were examined (Table 1, in which the crosses are listed in their order of analysis). These were sufficient to place 11 of the alleles in an unambiguous order (Fig. 1), built up in the following way using the two criteria outlined above.

All *sp am-1*^{x} lines were crossed with the *am-1*^{6} *his-1* line (DGC3651). All alleles map distally to *am-1*^{6}. The same *sp am-1*^{x} lines were then crossed with the *am-1*^{11} *his-1* stock (SM303). These results placed mutants *am-1*^{3} and *am-1*^{9} distal to *am-1*^{11} and all others proximal, except *am-1*^{8} whose site was unresolved.

All combinations of *am-1*^{3} *am-1*^{7} *am-1*^{8} and *am-1*^{9} were then tested to map the region distal to *am-1*^{11}, and to determine the sites of *am-1*^{7} and *am-1*^{8}. The unambiguous order of sites, proximal to distal, can be symbolized 8–7–3–9. Since *am-1*^{7} is proximal to *am-1*^{11}, the order 8–7–11–3–9 can be deduced.

Fincham (1967) obtained no prototrophs among 6 million progeny of crosses between *am-1*^{2} and *am-1*^{19}. Here, only two were found in 7·37 million viable ascospores. This frequency (0·027 per 10^5) is less than the 95% upper confidence limits obtained in corresponding homozygous crosses. However, *am-1*^{2} and *am-1*^{19} are apparently at different sites, since they result in alterations of different (but neigh-

bouring) amino acids (Wootton *et al.* 1973). Hence the site of *am-1*^{2} was placed proximal to *am-1*^{19} since this resulted in the excess of pD over Pd and of PD over pd, although neither of these excesses were significant.

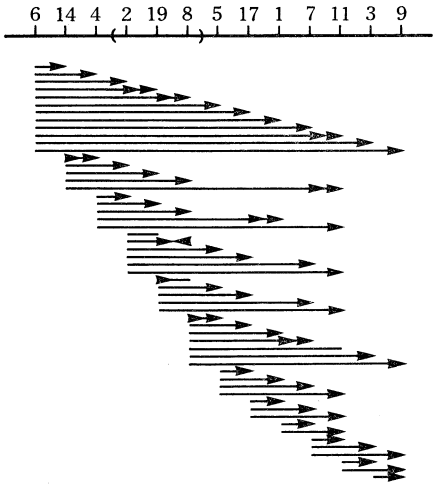


Fig. 1.—A map of the sites of 13 *am-1* alleles based on the flanking marker data from Table 1. Crosses between particular allele pairs are represented by a horizontal line between their sites. Arrows point from the proximal to the distal site as deduced from Criterion 1 (inner arrowheads pD > Pd) and from Criterion 2 (outer arrowheads PD > pd) (see text). An order is deduced only if differences between pD and Pd and between PD and pd are significant at the 5% level of probability.

Stocks of *am-1*^{2} and *am-1*^{19} were then crossed to all other *am-1* alleles whose sites mapped between *am-1*^{6} and *am-1*^{11}, except *am-1*^{1} with which complementation occurs. Without ambiguity, these crosses placed *am-1*^{14} and *am-1*^{4} proximal to, and *am-1*^{5}, *am-1*^{17}, and *am-1*^{7} distal to the sites of both *am-1*^{2} and *am-1*^{19}. However, *am-1*^{8} could not be placed unambiguously. Criterion 1 placed it distal to both *am-1*^{2} and *am-1*^{19}, but Criterion 2 placed it proximal to *am-1*^{2} and did not order it relative to *am-1*^{19}. Since Criterion 1 is an absolute criterion and Criterion 2 empirical, the former is considered to override the latter where a conflict arises. Hence *am-1*^{8} was tentatively placed distal to both *am-1*^{2} and *am-1*^{19}. However, the results of reciprocal crosses between *am-1*^{2} and *am-1*^{8} and between *am-1*^{19} and *am-1*^{8} were heterogeneous at the 5% level, implying significant genetic differences between the stocks. The definite position of *am-1*^{8} will therefore have to await further study of this variation.

The allele *am-1*^{14} was then mapped proximal to *am-1*^{4}. Both were tested with *am-1*^{8} and found to be proximal to it. The region between *am-1*^{2}, *am-1*^{19}, and *am-1*^{11} was then mapped. An unambiguous order of sites, proximal to distal, of 8–5–17–7 was obtained. Finally *am-1*^{1} was crossed to all alleles between *am-1*^{6} and *am-1*^{11}, apart from *am-1*^{14}, *am-1*^{2}, and *am-1*^{19}, each of which it complements strongly. The *am-1*^{1} allele was placed unambiguously between *am-1*^{17} and *am-1*^{7}.

(*c*) *General Pattern of Recombination in* am-1 *in* rec-3 *Background*

Thirteen sites of allelic difference in *am-1* have been ordered almost without ambiguity. The only uncertainty is in the relative order of alleles *am-1*^{2}, *am-1*^{19}, and *am-1*^{8} in the region between alleles *am-1*^{4} and *am-1*^{5}. Using the most likely order, trends and patterns of recombination in *am-1* can be followed.

Figure 2 summarizes the flanking marker data from Table 1 in a form modified from that proposed by Whitehouse and Hastings (1965). The pairs of alleles crossed are indicated on the left of the figure in an order which will indicate any trends across the locus dependent on the site of the more proximal allele. The data have not been corrected for additional crossing-over between the *sp* and *his-1* loci independent of recombination in *am-1*. Results from different crosses between the same pair of *am-1*$^{2}\times$*am-1*^{19} omitted.

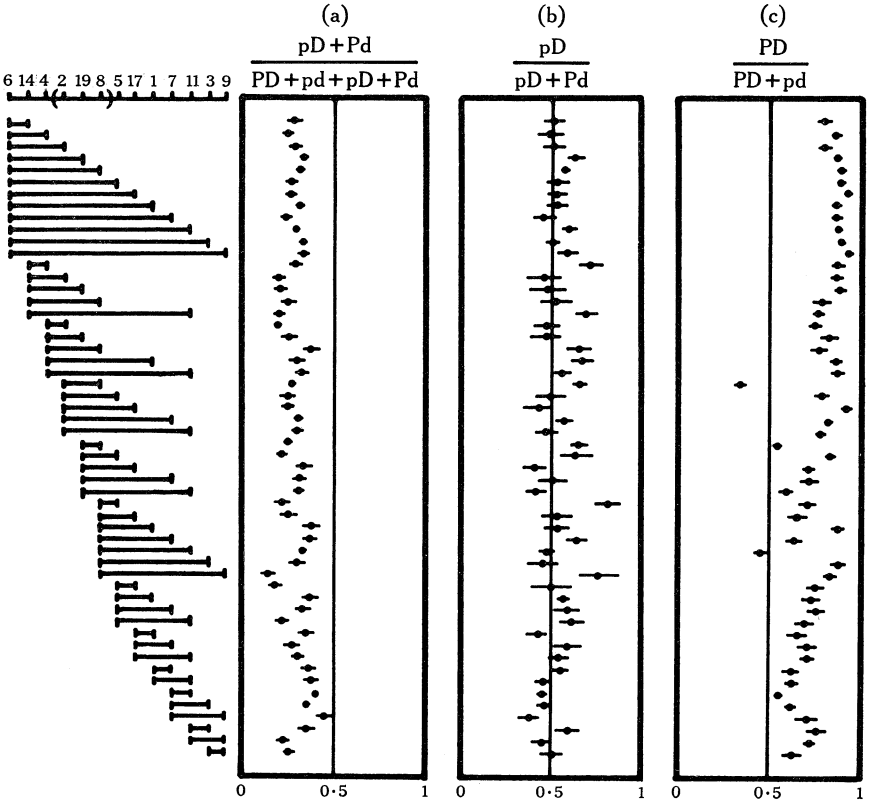


Fig. 2.—Flanking marker results from Table 1 for crosses between *am-1* alleles in *rec-3* background. The particular alleles crossed are indicated on the left by horizontal lines between their sites. The order chosen will demonstrate any significant trends across the locus dependent on the position of the proximal site. PD, pd, pD, and Pd represent the four classes of flanking markers among *am-1*$^{+}$ progeny. The parent carrying the proximal allele also carries the markers PD. The horizontal bars at each point extend for 1 S.E. of the estimate in each direction (calculated using the binomial distribution).

Figure 2(*a*) shows the proportion of prototrophs recombinant for flanking markers. This ranges from 12·5 to 44·2%, with a mean of 29·5%. There is more variation than can be attributed to chance, but it is not obviously associated with map position. Figure 2(*b*) shows the pD class as a proportion of the recombinant classes (pD+Pd). It ranges from 37·7 to 80·8% with a mean of 53·3%. It is never

significantly less than 50%, because Criterion 1 assigns pD to the larger of these two classes. Here, also, the variation is not obviously associated with map position, and is more likely due to background genetic variation.

In contrast, Figure 2(*c*), which plots the proportion of the two classes with parental associations of flanking markers which are PD (Criterion 2), does show a significant trend. As the more proximal site moves from proximal to distal ends of the *am-1* locus, the excess of PD over pd decreases. PD prototrophs are assumed to arise from conversion to wild type of the more proximal allele without concurrent crossing-over, and pd from conversion of the more distal allele. Hence a gradient in conversion frequencies occurs, with more conversion always occurring at the more proximal site, but with the excess being less if both sites are in the distal region of the locus. Thus Criterion 2 (PD > pd) can be validly used for mapping *am-1*, since no reversal of polarity has been shown across the whole *am-1* locus, though a reduction in the strength of polarity occurs at the distal end. The only cross where pd is significantly larger than PD is between *am-1*^{2} and *am-1*^{8}, the cross in which Criterion 1 and Criterion 2 gave discordant results. Criterion 1 was considered to override Criterion 2, and hence PD was of necessity less than 50% for this cross.

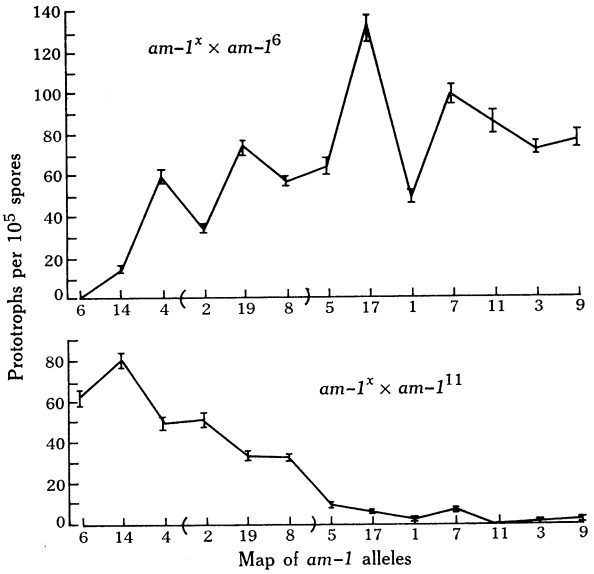


Fig. 3.—Prototroph (*am-1* $^+$) frequencies from Table 1 for *rec-3* crosses between 13 *am-1* alleles and *am-1*^{6} (upper graph) and *am-1*^{11} (lower graph), plotted in the order of their sites from Figure 1. The vertical bars at each point extend for 1 S.E. of the estimate in each direction.

To this point, only flanking marker data have been considered. We can now examine prototroph frequencies (Table 1) in relation to the order of sites obtained from flanking markers. Figure 3 shows these frequencies from crosses between all alleles and *am-1*^{6} (upper curve) and *am-1*^{11} (lower curve) in relation to map order of the alleles. A general trend of increased frequency with increased separation of the sites is apparent, especially with *am-1*^{11} crosses. However, the steady increase is broken by several fluctuations, signifying inconsistencies in the prototroph frequency map. In *am-1*^{6} crosses, frequencies apparently plateau with alleles distal to *am-1*^{4}.

Figure 3 also shows that crosses between distal alleles often give frequencies relatively lower than those between proximal alleles, an observation also apparent in the other results in Table 1. For example those crosses made between alleles distal to, and including, *am-1*^{5} yield a *maximum* frequency of 13·7 per 10^5, whereas crosses involving the two most proximal alleles *am-1*^{6} and *am-1*^{14} yield a *minimum* frequency of 13·4 prototrophs per 10^5 offspring. It is of interest that this higher prototroph frequency with proximal alleles is associated with the relatively higher conversion frequencies in the proximal region of *am-1* [Fig. 2(*c*)].

(*d*) *Comparison with Fincham's Map*

The map obtained here is in reasonable agreement with that which Fincham (1967) constructed using nine *am-1* alleles, comprising those used here save 8, 9, 11, and 17. The following disagreements are shown. (1) The relative orders of the sites of *am-1*^{1}, *am-1*^{3}, and *am-1*^{7} are different. Fincham's order is one of several possible from his results, and another of them agrees with the unambiguous order my data prescribe. (2) Fincham's map is inverted relative to the centromere. When Criterion 1 is applied to Fincham's data, a direction opposite to mine is obtained, whereas Criterion 2 gives concurrent directions. This difference may be due to genetic differences between our stocks. Alternatively, the conflict may result from our use of different distal markers. Fincham used alleles at the *inositol* locus, which is three times further from *am-1* than is *his-1*, the marker locus used here (9 units versus 3 units). More cross-overs, independent of recombination in *am-1*, would therefore occur in this region in Fincham's experiments. They would boost the Pd class relative to pD, since Pd prototrophs would arise from crossing-over among the prototrophs which would otherwise have been the more frequent non-cross-over class, PD.

The prototroph frequencies obtained by Fincham are in roughly the same relative magnitude as the *rec-3* frequencies obtained here, but are at least eight times lower in absolute magnitude. This confirms Catcheside's (1968) suggestion that all of Fincham's crosses involved *rec-3*$^{+}$. Fincham also lists nine crosses between *am-1* alleles which I did not make. For seven of them, Criterion 2 can be based on a significant difference, and the order deduced in each case is consistent with that shown in Figure 1.

IV. Effects of *rec-3*$^{+}$

(*a*) *Results*

Now that an almost unambiguous order of 13 sites in *am-1* has been obtained in *rec-3* background, the effect of *rec-3*$^{+}$ can be better examined across the *am-1* map. Only crosses between *am-1*^{6} and the other 12 alleles will be considered. Twelve *sp am-1*^{x} lines of *rec-3*$^{+}$ genotype were crossed to the same *am-1*^{6} *his-1* tester (DGC3651) used in the *rec-3*×*rec-3* mapping crosses. The *rec-3*$^{+}$ gene is dominant (Catcheside 1966*a*), and any effect should show in such heterozygous crosses. Table 2 shows the frequency and flanker distribution among prototrophs of these *rec-3*$^{+}$× *rec-3* crosses.

Prototroph frequency is reduced 9·6- to 36-fold (Fig. 4), with possibly more reduction in the proximal region. However, care should be taken in the interpreta-

tion of these relative reductions, each based on two crosses only. Prototroph frequencies are quite variable between different lines of the same *rec-3* constitution, and more crosses need to be made using different stocks to obtain a measure of this variation.

TABLE 2

FREQUENCIES AND DISTRIBUTIONS OF FLANKING MARKERS OF *am-1*$^+$ PROGENY FROM CROSSES BETWEEN *rec-3*$^+$ LINES OF *sp am-1*^{x} + CONSTITUTION AND A *rec-3* TESTER, + *am-1*^{6} *his-1*

Parents		Frequency of *am-1*$^+$ progeny per 10^5 spores	S.E.	Distribution of flanking markers of *am-1*$^+$ progeny				
am-1^x *sp* +	*am-1*^{y} + *his-1*			*sp* +	+ *his*	+ +	*sp his*	Total
14	6	0·37	0·031	35	46	20	24	125
4	6	2·5	0·16	52	129	40	19	240
2	6	1·8	0·11	23	137	55	47	262
19	6	3·6	0·24	48	134	23	35	240
8	6	2·8	0·15	68	152	43	57	320
5	6	2·9	0·17	56	117	61	33	267
17	6	5·3	0·28	41	134	48	53	276
1	6	3·7	0·21	40	125	49	26	240
7	6	8·9	0·45	122	143	59	62	386
11	6	8·8	0·56	55	90	45	50	240
3	6	3·0	0·12	51	437	85	102	675
9	6	5·9	0·39	26	142	41	46	255

The effect of *rec-3*$^+$ on flanking marker distribution is shown in square root charts (Fig. 5). The comparison between pD and Pd classes [Fig. 5(*b*)] and the PD+pd and pD+Pd classes [Fig. 5(*c*)] show a small amount of significant variation, but it is not associated with the *rec-3*/*rec-3*$^+$ difference. In contrast, the relative proportion of the two parental classes PD and pd show marked differences correlated with *rec-3* versus *rec-3*$^+$ [Fig. 5(*a*)].

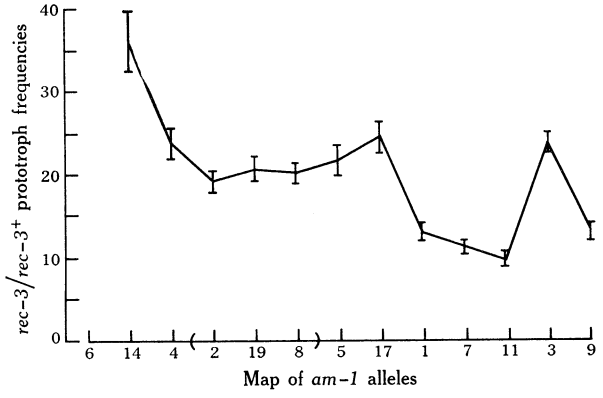


Fig. 4.—Ratios of prototroph frequencies from crosses between 12 *am-1* alleles and *am-1*^{6} in *rec-3* × *rec-3* background (from Table 1) compared with equivalent crosses in *rec-3*$^+$ × *rec-3* background (from Table 2). The ratios are plotted in the order of the sites of *am-1* alleles used (Fig. 1). The vertical bars extend for 1 S.E. in each direction.

In Figure 6, the proportion of these parental classes which is PD is shown for *rec-3* and *rec-3*$^+$ crosses in relation to the site of the *am-1* allele crossed with *am-1*^{6}. This proportion probably represents the relative frequency of conversion at the more

proximal site. In *rec-3* crosses, conversion of the proximal *am-1*^{6} allele apparently increases at the expense of conversion of the distal allele, as the site of the latter moves further and further from *am-1*^{6}, since PD shows a relative increase.

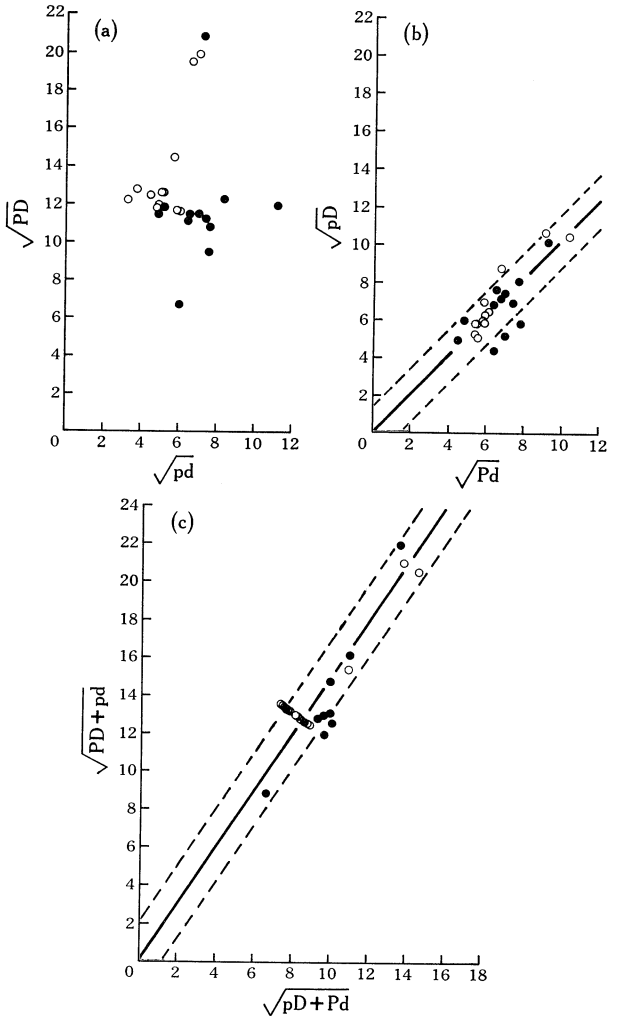


Fig. 5.—Square root charts of the distributions of flanking markers among *am-1*$^+$ progeny of crosses between 12 different *sp am-1*^{x} + lines (one for each allele except *am-1*^{6}) and a + *am-1*^{6} *his-1* tester. In this figure PD = + *his-1*, pd = *sp* +, pD = *sp his-1*, and Pd = + +, since *am-1*^{6} is the most proximal allele. o *rec-3* × *rec-3* results from Table 1; • *rec-3*$^+$ × *rec-3* results from Table 2. In (*b*) and (*c*), the pooled means are shown by solid lines and the 95% confidence limits by dotted lines.

When *rec-3*$^+$ is present, this proximal preference is reduced in nearly every case, shown by the significant reduction in PD relative to pd. However, the proximal allele still holds the preference, since PD never falls below 50%. There is no obvious trend in the extent of reduction brought about by *rec-3*$^+$ across the map. The varia-

tion in the level of reduction, and its absence in *am-1*^{6}×*am-1*^{2} and *am-1*^{6}×*am-1*^{3} crosses, may reflect other genetic factors in the stocks used. More crosses are needed to pinpoint these differences more closely.

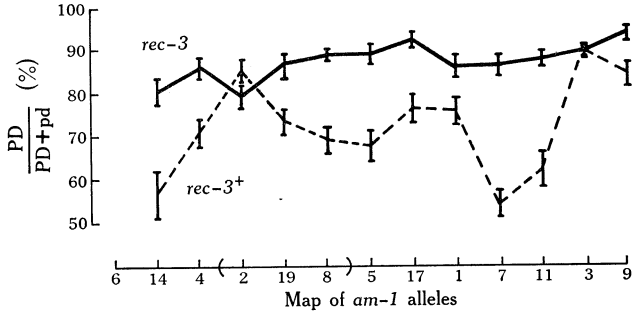


Fig. 6.—Effect of *rec-3*$^{+}$ on the relative proportion of those prototrophs not showing crossing-over from crosses between 12 *am-1* alleles and *am-1*^{6}. The proportion is shown as the percentage of such prototrophs with the same combination of flankers as entered the cross with the more proximal allele (in this case *am-1*^{6}). The percentages are plotted in the map order of the sites of the *am-1* alleles used (Fig. 1). The *rec-3* × *rec-3* results are from Table 1 and equivalent *rec-3*$^{+}$ × *rec-3* results from Table 2. The vertical bars at each point extend for 1 S.E. of the estimate in each direction.

(*b*) *Discussion*

The results reported here confirm and extend previous observations of the influence of *rec-3* on allelic recombination in *am-1*. The observed reduction in prototroph frequency brought about by *rec-3*$^{+}$ (Catcheside 1968) has been shown to occur for all 13 alleles tested. The change which *rec-3*$^{+}$ causes to polarity of conversion of *am-1* alleles 6 and 11 (i.e. PD relative to pd) (Smyth 1971) has now been observed with nine other alleles. It seems that mapping the *am-1* alleles has allowed the detection of a gradient of conversion frequency which probably reaches a maximum at a point proximal to the site of *am-1*^{6}, the most proximal allele mapped. The steepness of this gradient is apparently reduced in the presence of *rec-3*$^{+}$.

The lack of effect of *rec-3*$^{+}$ on the relative proportion of total prototrophs showing crossing-over (pD+Pd) (Smyth 1971) is also apparently consistent over all of the *am-1* locus. Since *rec-3*$^{+}$ reduces the absolute number of this class to the same extent as the sum of those prototrophs not showing crossing-over (PD+pd), it seems that these two classes are the common consequence of one recombination process which is sensitive to control by *rec-3*$^{+}$. It has been suggested that *rec-3*$^{+}$ represses the initiation of recombination at a site proximal to *am-1*^{6}, thus reducing the entry of hybrid DNA into *am-1* from this site (Catcheside 1968; Smyth 1971). The observed change in polarity could result from residual entry of hybrid DNA into *am-1* from another site distal (Smyth 1971) or more proximal (Whitehouse 1972) to the locus. The present results are consistent with these speculations.

It is now apparent why my *rec-3* map and the *rec-3*$^{+}$ map of Fincham (1967) give a similar order of sites. Prototroph frequencies are *all* reduced by *rec-3*$^{+}$, and

the possible larger reduction which occurs proximally does not affect the *order* of sites. Also, *rec-3*$^+$ apparently does not affect the proportion of pD relative to Pd on which Criterion 1 is based. While *rec-3*$^+$ does reduce the proportion of PD relative to pd (Criterion 2), PD remains in excess for all combinations tested. The same order is therefore deduced from *rec-3* and *rec-3*$^+$ data.

V. General Discussion

Allele-specific effects are noticeably absent from the present results. Instead, the recombination behaviour of the 13 alleles seems to correlate well with the order of their sites. Recent data from *Ascobolus* show that conversion patterns of mutants depend markedly on the mutagen used to obtain them, presumably through the specific types of base change induced (Leblon 1972). In particular, mutants which are due to a frame shift showed an excess of conversions from wild type to mutant, whereas those due to base substitutions apparently resulted in more mutant to wild-type converts. The latter would yield many more prototrophs in the present experiments. It is therefore possible that the *am-1* mutants used here are all base substitutions. Based on amino acid replacements, mutant *am-1*^{2} *is* such a substitution; and mutant *am-1*^{19} can be further classified as an A-T or T-A transition (Wootton *et al.* 1973). The *am-1* mutants 1, 2, 5, 6, 7, 8, 9, and 11 were all induced by ultraviolet light (Fincham 1959) and this agent is thought to induce a majority of base substitutions, although some base additions and deletions also seem to result (Kilbey *et al.* 1971). Nitrous acid is apparently more specific in the induction and reversion of base substitutions (Malling and de Serres 1967). Mutants *am-1*^{14}, *am-1*^{17}, and *am-1*^{19} were induced with nitrous acid (Fincham and Stadler 1965), and these mutants, as well as *am-1*^{1}, *am-1*^{4}, and *am-1*^{5}, were also caused to revert with it (Seale 1968).

The dependence of conversion pattern on the type of base change involved can be explained by proposing differences in correction of various classes of base mismatch in double helical DNA. Such mismatching would result when hybrid DNA associated with recombination covers a site of heterozygosity. When two heterozygous sites are close, these supposed correction events seem to cover them both, using only one of the DNA chains of the hybrid as a template (Fogel and Mortimer 1969). When the sites represent mutants in repulsion phase (as here), such correction cannot lead to wild-type recombinants (prototrophs). This explains, to some extent, why such prototroph frequencies are a function of the physical separation of sites. Mutants *am-1*^{2} and *am-1*^{19}, whose sites of difference from normal and one another are only four nucleotides apart (Wootton *et al.* 1973), yield prototrophs no more frequently than revertants. Thus it seems that correction events rarely begin or end between these two particular sites.

Since gene and polypeptide are collinear, the localization in the *am-1* map of the sites of mutants with similar altered properties should reflect the regions of the polypeptide concerned with control of these properties. Several separate regions of the map would be expected, as regions controlling particular functions probably involve several different amino acid sequences brought into close proximity by specific folding and by aggregation of the six identical polypeptide chains of the active enzyme (Wootton *et al.* 1973).

The products of *am-1* mutants have largely been studied in the active enzyme formed by aggregation of individually defective monomers from complementing pairs of mutants (summarized by Fincham 1968). Figure 7 relates the present

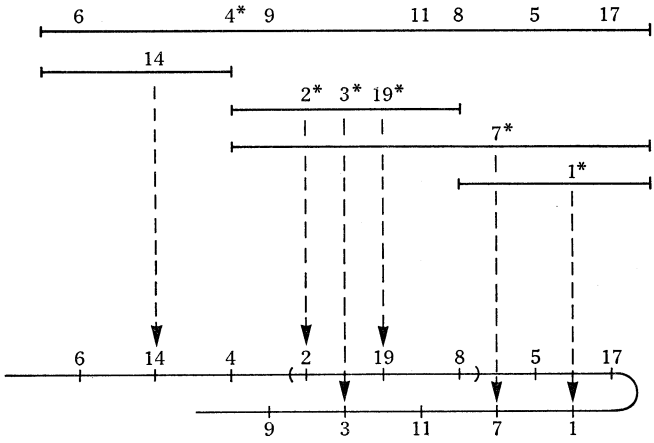


Fig. 7.—A complementation map of 13 *am-1* mutants from Fincham and Stadler (1965) (above), related to the genetic map of the sites of these mutants from Figure 1 (below). The genetic map has been folded near its mid-point. This yields the simplest geometric correspondence between the two maps. Asterisked mutants are those which produce protein cross-reacting with antisera to the wild-type *am-1* protein, glutamate dehydrogenase (Roberts and Pateman 1964).

genetic map to one of the two linear complementation maps possible from the results of Fincham and Stadler (1965). To obtain the simplest geometric correspondence, the genetic map has been folded near its midpoint. One interpretation of the segments of the complementation map is that they represent different functional regions of the glutamate dehydrogenase polypeptide. Each segment would thus contribute to the properties which are altered in the mutants involved in it. Thus the segment involving *am-1*^{14} would be concerned in a site of association of monomers, as this mutant probably produces monomers unable to aggregate by themselves. The segment containing mutants *am-1*^{2}, *am-1*^{3}, *am-1*^{19}, and *am-1*^{7} would be involved in the maintenance of conformation, as these mutants probably produce a monomer whose defective conformation can be corrected if it aggregates with a proportion of certain other monomers. As expected, this group of mutants specify a protein which cross-reacts with antibody to purified wild-type enzyme (Fig. 7). Mutants *am-1*^{2}, *am-1*^{3}, and *am-1*^{19} affect only this segment. They probably have catalytic sites which are potentially functional, since they show activity under special conditions *in vitro*. In contrast, Fincham (1968) has suggested that mutants in the third segment (*am-1*^{1} and *am-1*^{7}) *are* involved in amino acid substitutions at the catalytic site. They have not shown activity *in vitro*, and they apparently revert only by true back mutation. Mutant *am-1*^{4} resembles these two in reversion properties, and like them, it produces protein homologous to glutamate dehydrogenase. If the site of difference of *am-1*^{4} is involved in the catalytic centre, it would probably be brought close to the sites of *am-1*^{6} and *am-1*^{7} by specific folding of the polypeptide chain.

The remaining six mutants are non-complementing, and do not produce cross-reacting protein. Of these, at least *am-1*^{5} and *am-1*^{17} can be suppressed by specific suppressors at unlinked loci, suggesting that they are nonsense mutants (Seale 1968). Their genetic position suggests that, unlike chain-terminating mutants of the gene specifying β-galactosidase in *Escherichia coli* (Langridge and Campbell 1969), they are not close to one end of the locus.

The genetic map of *am-1* mutants obtained here should help in the sequencing of wild type and mutant glutamate dehydrogenase already begun (Wootton *et al.* 1973). Together with further biochemical characterization of the various forms of the enzyme, these studies should lead to a complete understanding of the mechanism of action of the enzyme from wild-type *Neurospora crassa* and from pairs of complementing mutants.

VI. Acknowledgments

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