EFFECT OF *rec-3* ON POLARITY OF RECOMBINATION IN THE *amination-1* LOCUS OF *NEUROSPORA CRASSA*

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Summary

Crosses between *am-1* alleles 6 and 11 produce *am-1*$^+$ recombinants (prototrophs) eightfold less frequently in the presence of *rec-3*$^+$, an unlinked dominant gene. A factor also controls the relative proportions of the two classes of prototrophs with parental combinations of the flanking markers *spray* (5 units proximal) and *histidine-1* (3 units distal). Those prototrophs with the same combination as the *am-1*^{6} parent are reduced from 81 to 59% of the two classes. This factor segregated in complete association with *rec-3*$^+$ in 12 offspring of a *rec-3* × *rec-3*$^+$ cross. It is probably *rec-3*$^+$ itself.

Rec-3$^+$ apparently affects polarity by reducing recombination (which includes conversion) more in the proximal (*am-1*^{6}) region of *am-1* than the distal (*am-1*^{11}) region. The *rec-3*$^+$ gene product might reduce the level of hybrid DNA which spreads across *am-1* from a proximal recognition site, without affecting a smaller level originating distally.

I. INTRODUCTION

Genes which influence recombination occur naturally in several laboratory stocks of *Neurospora crassa* (Catcheside 1968; Catcheside and Austin 1969). They control the frequency of recombination at specific localities in the genome, but are not closely linked to the region affected. The dominant allele reduces recombination about 5–40-fold. No effects apart from those on recombination have been observed.

These properties are consistent with the *recombination* (*rec*) genes having a regulatory function. The products of the *rec*$^+$ alleles are thought to be active in reducing the level of recombination initiated at specific recognition sites (Angel, Austin, and Catcheside 1970).

Rec-1$^+$ not only reduces the frequency of allelic recombination at the *histidine-1* (*his-1*) locus (Jessop and Catcheside 1965), but also affects its polarity (Catcheside 1968; Thomas and Catcheside 1969). Polarity is the apparent tendency for recombination to occur preferentially in certain regions of a locus. When selected recombinants are studied, it may be judged by following the regions flanking the locus under study (Murray 1969).

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In fungi, a cross is made between stocks of genotype P m^1 + D and p + m^2 d, where P, p and D, d are markers at closely linked loci respectively proximal and distal to that of the allelic mutants m^1 and m^2. The site of difference of m^1 is proximal to that of m^2. Wild-type recombinants between m^1 and m^2 are selected from the haploid offspring, and the distribution of markers among them determined. Where the ++ recombinants have been obtained in asci along with the other three meiotic products, those with flanking markers PD indicate conversion of m^1 to + without concurrent crossing over, whereas pd are usually associated with conversion of m^2 to + (Stadler and Towe 1963; Fogel and Hurst 1967).

Rec-1$^+$ changes the relative proportions of these two classes, reducing PD from 60 to 40% of the total (Thomas and Catcheside 1969). Therefore it can be argued that *rec-1*$^+$ causes a relatively larger reduction in conversion of mutant to wild type at the more proximal of two sites, and thus alters the polarity of recombination in *his-1*.

These effects have been interpreted in terms of hybrid DNA hypotheses of recombination (Holliday 1968; Whitehouse 1969). It has been suggested (Whitehouse 1966; Catcheside 1968; Thomas and Catcheside 1969) that *rec-1*$^+$ greatly reduces the level of hybrid DNA which extends into *his-1* from the proximal direction, without changing a much smaller level entering from the distal side.

This work investigates whether *rec-3*$^+$, which affects the frequency of recombination in the *amination-1* (*am-1*) locus (Catcheside 1966, 1968), also affects the polarity.

II. Methods

(*a*) *Mutants Used*

Mutants employed during the study are listed in the following tabulation:

Locus	Mutant	Original Reference
am-1	6 (K314)	Fincham (1959)
	11	Fincham (1959)
sp	B132	Perkins (1959)
his-1	K83	Catcheside (1960)
cot-1	C102t	Mitchell and Mitchell (1952)
arg-3	K125	Catcheside (unpublished data)

The *colonial temperature sensitive-1* (*cot-1*) mutant was bred into stocks to facilitate handling. All mutants were from Professor D. G. Catcheside's collection, except *am-1*^{11} which was obtained from the Fungal Genetics Stock Center, Dartmouth College, Hanover, New Hampshire 03755, U.S.A. (F.G.S.C. No. 790.)

(*b*) *Media*

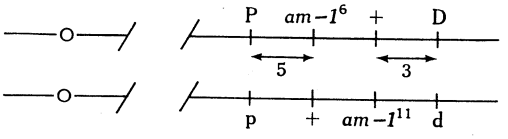
The basic growth medium for all purposes save crosses was Vogel's (1964) medium N. For maintaining cultures in tubes it was solidified with Ionagar No. 2 (Oxoid, 1·2 g/100 ml) and 2% sucrose added. For other purposes Bactoagar (Difco, 2 g/100 ml) was used, and the following carbon sources provided: 0·5% sorbose + 0·1% sucrose for testing constitutions on plates, except for tests of the morphological mutant *spray* (*sp*) where 0·25% sorbose + 1% sucrose allowed better discrimination; 0·5% sorbose + 0·0125% glucose + 0·025% fructose for germinating ascospores on plates.

Crosses were made using tubes of liquid Westergaard and Mitchell's (1947) medium containing 2% sucrose. Folded filter paper dipped into the liquid and acted as an inert substrate for perithecia. Parents were added together as conidial suspensions, and crosses incubated at 25 ± 1°C for at least 4 weeks.

Supplements required for growth of mutants were added at the following rates: 450 mg/l L-alanine for *am-1*; 300 mg/l L-histidine.HCl for *his-1*, but 200 mg/l if L-alanine also present; either 500 mg/l L-arginine.HCl or 200 mg/l L-citrulline for *arginine-3* (*arg-3*). To inhibit the leaky growth of *am-1* mutants, 1500 mg/l L-glycine was added (Pateman 1957).

(c) *Assay of* am-1$^+$ *Recombinants*

Recombination in *am-1* was studied using crosses of the type:



where P and p represent alleles at the *sp* locus, and D and d alleles of *his-1*. The map order and distances between loci are from Smyth (1970).

The frequency of *am-1*$^+$ recombinants among the haploid offspring was determined by a layer-plating technique similar to that of Catcheside (1966). However, the selective plates contained glycine, the non-selective plates alanine, histidine being added for crosses segregating at *his-1*. The supplements used for the *arg-3* mutant interfered with the assay, so for crosses involving this mutant, only *arg-3*$^+$ progeny could be tested. Arginine supports the growth of *am-1* mutants (Fincham 1950) and the sample of citrulline used was found to increase the already leaky growth of *am-1* mutants while allowing only slow growth of *arg-3*; *am-1*$^+$ spores. These two growth forms were indistinguishable on selective plates.

To record the constitution of *am-1*$^+$ recombinants at the flanking loci, two small pieces of each were inoculated on suitable tester plates.

By using serial dilutions of a spore suspension, the assay was shown to give homogeneous results over the range $0{\cdot}90 \times 10^5$–$22{\cdot}6 \times 10^5$ viable spores screened in 15 ml of suspension. Also, the results were unaffected by use of different vegetative subcultures for crosses, environmental conditions experienced by crosses set up on different occasions, and differences in the experimenter's performance on different days of assay.

III. Results

(a) *Contribution of Reversion to Prototrophs*

No prototrophs were obtained in 4·8 million offspring of *am-1*^{11} × *am-1*^{11} crosses. The 5·9 million spores tested from *am-1*^{6} × *am-1*^{6} crosses yielded three prototrophs which were probably revertants. The upper limit, below which prototroph frequency in these homozygotes lies with 95% certainty, is 0·063 prototrophs per 10^5 viable spores for *am-1*^{11} crosses, and 0·132 per 10^5 spores for *am-1*^{6}.

Therefore reversion probably contributes negligibly to prototrophs from *am-1*^{6} × *am-1*^{11} crosses, since the lowest frequency observed was 5·24 per 10^5 viable spores.

(b) *Differential Viability of Flanking Markers*

To determine whether *sp* and *his-1* influence viability of prototrophs, an orthogonal set of crosses homozygous for *rec-3* was analysed (Table 1). Crosses of the same genotype are homogeneous, but the four different types of cross are highly heterogeneous ($\chi^2 = 29{\cdot}66$, 9 d.f., $P < 0{\cdot}1\%$). However, this heterogeneity does not result from viability differences. Expected frequencies of *am-1*$^+$ recombinants of each genotype were calculated assuming equal viability by apportioning the grand

totals of the PD, pd, pD, and Pd classes among the four types of cross in proportion to the total in each. The observed values fit these (Table 1) ($\chi^2 = 4{\cdot}323$, 3 d.f., $20\% < P < 30\%$), so other sources of heterogeneity must be responsible.

TABLE 1

CROSSES BETWEEN *am-1*^{6} AND *am-1*^{11} USING ALL FOUR POSSIBLE COMBINATIONS OF FLANKING MARKERS IN THE PARENTS

Parents				No. of Crosses	Heterogeneity			Flanking Markers of *am-1*$^+$ Progeny				Total
P *am-1*^{6}	D	p *am-1*^{11}	d		χ^2	D.F.	*P*	PD	pd	pD	Pd	
sp	+	+	*his-1*	5	15·48	12	20–30%	1117	153	217	193	1680
+	*his-1*	*sp*	+	5	5·31	12	90–95%	1000	155	286	239	1680
+	+	*sp*	*his-1*	4	9·64	9	30–50%	632	71	142	115	960
sp	*his-1*	+	+	4	12·54	9	10–20%	585	97	163	115	960
Total								3334	476	808	662	5280
								sp +	+ *his-1*	+ +	*sp his-1*	
Total of specific genotypes								1529	1431	1185	1135	5280
Expected total, equal viability								1479·5	1479·5	1160·5	1160·5	5280

Square root charts comparing the two parental combinations [Fig. 1(*a*)] and the two non-parental classes [Fig. 1(*b*)] show no heterogeneity. However, the pooled

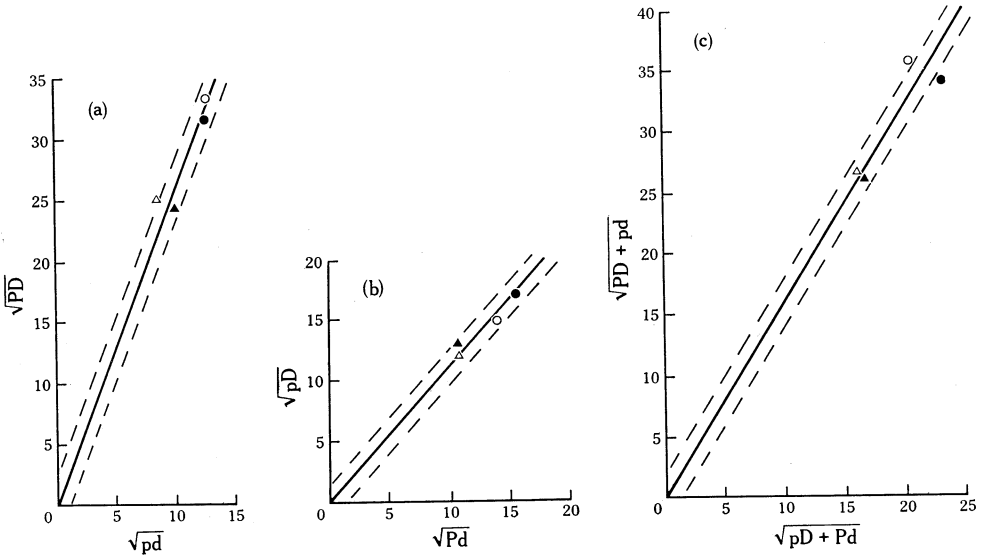


Fig. 1.—Square root charts of the distributions of flanking markers among *am-1*$^+$ recombinants of Table 1. Crosses are of the type P *am-1*^{6} D × p *am-1*^{11} d, where PD and pd are respectively *sp* + × + *his-1*(○), + *his-1* × *sp* + (●), + + × *sp his-1*(△), and *sp his-1* × + + (▲). Chart (*a*) shows the two *am-1*$^+$ classes with parental combinations of markers, chart (*b*) those with non-parental combinations, and chart (*c*) compares parental and non-parental combinations. The solid line on each chart is the mean, and the dashed lines are 95% confidence limits.

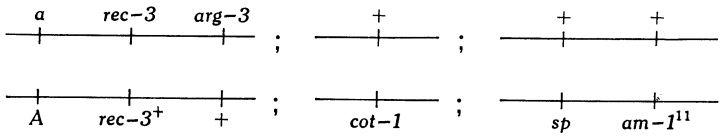
parental and pooled non-parental classes show two points outside the 95% confidence limits [Fig. 1(*c*)]. The origin of these differences is not known, but since stocks used

in each type of cross were much more closely related to each other than those in other crosses, it is likely that inherited differences with small effects are involved.

(c) *Effect of* rec-3$^+$ *on Distribution of Flanking Markers*

Preliminary results indicated that *rec-3* and *rec-3*$^+$ stocks differed in the representation of distal markers among *am-1*$^+$ recombinants (Catcheside 1968). Using markers on both sides of *am-1*, it appeared that this difference was restricted to the parental classes PD and pd. To test if this difference was due to *rec-3*$^+$ and not to some closely linked factor, an attempt was made to separate the two determinants among progeny of a *rec-3* × *rec-3*$^+$ cross.

Rec-3 is in linkage group I between the mating type and *arg-3* loci (Catcheside 1968). By selecting recombinants between these two loci, the chance of separating the hypothetical factor from *rec-3* is increased. Therefore the following cross was prepared:



In all, 38 of 120 *arg-3* progeny were *A* following crossing-over between mating type and *arg-3*. The map distance is therefore 31·7±4·2 units and is unusually large for this interval (B. Austin and D. G. Catcheside, unpublished data). Twenty-three of these recombinants, of *A arg-3*; *cot-1*; *sp am-1*^{11} genotype, were then tested by crossing to an *a rec-3*; *cot-1*; *am-1*^{6} *his-1* line.

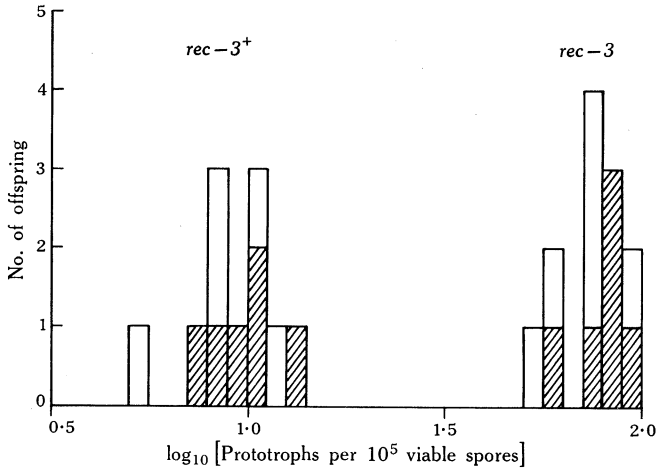


Fig. 2.—Distributions of prototroph (*am-1*$^+$ recombinant) frequencies of 23 *am-1*^{11} sibs from a *rec-3* × *rec-3*$^+$ cross tested with an *am-1*^{6} line. Flanking markers were determined for crosses indicated with cross-hatching.

Prototroph (*am-1*$^+$) frequencies from these crosses are discontinuously distributed about two modes (Fig. 2). The 11 low-yielding lines (with a weighted mean

frequency of $9 \cdot 35 \pm 0 \cdot 64$ prototrophs/10^5 viable spores) are *rec-3*$^+$; the other 12, with a mean of $76 \cdot 3 \pm 3 \cdot 8$ per 10^5 spores, are *rec-3*. Thus 8·2 times more recombinants are obtained from *rec-3* crosses on the average. In all, 12 out of 23 crossovers between mating type and *arg-3* were distal to *rec-3*, the remainder proximal. Hence *rec-3* is $16 \cdot 5 \pm 4 \cdot 0$ map units proximal to mating type and $15 \cdot 1 \pm 3 \cdot 9$ units distal to *arg-3*.

Flanking markers were recorded among *am-1*$^+$ recombinants from the first six *rec-3* and the first six *rec-3*$^+$ progeny obtained. The *rec-3* results are homogeneous at the 5% level and the *rec-3*$^+$ results at the 1% level (Table 2). The only significant

TABLE 2

DISTRIBUTIONS OF FLANKING MARKERS FROM SIX *rec-3* AND SIX *rec-3*$^+$ SIBS OF *sp am-1*^{11} CONSTITUTION CROSSED WITH A *rec-3*; *am-1*^{6} *his-1* TESTER

	rec-3 Sibs					*rec-3*$^+$ Sibs			
	am-1$^+$ Recombinant Progeny					*am-1*$^+$ Recombinant Progeny			
	+ *his-1* (PD)	*sp* + (pd)	*sp his-1* (pD)	+ + (Pd)		+ *his-1* (PD)	*sp* + (pd)	*sp his-1* (pD)	+ + (Pd)
	136	18	34	52		105	60	40	34
	115	34	43	48		90	59	48	43
	126	37	39	38		104	47	41	48
	119	33	36	52		71	59	53	57
	142	29	33	36		80	70	44	46
	128	28	41	43		70	64	49	57
Totals	766	179	226	269	Totals	520	359	275	286
	χ^2(heterogeneity) = 18·97, 15 d.f., $20\% < P < 30\%$					χ^2(heterogeneity) = 29·12, 15 d.f., $1\% < P < 2\%$			

difference between the sets is in the two parental classes PD and pd (Fig. 3). All *rec-3* × *rec-3* crosses give a significantly higher proportion of PD than all *rec-3* × *rec-3*$^+$ crosses.

A factor controlling a lower proportion of PD to pd has therefore segregated in unbroken association with *rec-3*$^+$ in 12 offspring of a *rec-3* × *rec-3*$^+$ cross. It is probably identical to the *rec-3*$^+$ gene. However, assuming it is not, the distance from *rec-3* within which its locus lies with 95% surety is 7 map units, calculated as follows. If p is the probability of crossing-over between *rec-3* and the hypothetical locus, $p/0 \cdot 317$ is the proportion of crossovers between mating type and *arg-3* (31·7 units apart) which would occur in this region. The probability of observing none out of 12 is $(1 - p/0 \cdot 317)^{12}$. If this is taken as 5%, then $p = 0 \cdot 070$.

IV. DISCUSSION

Rec-3$^+$ probably has pleiotropic effects on frequency and polarity of recombination in *am-1*. Alternatively, two factors may be involved which are less than 7 map units apart by chance. However, Thomas and Catcheside (1969) were also unable

to separate from *rec-1*$^+$ a factor with exactly analogous effects on polarity of recombination in *his-1*. The chance of two such cases of fortuitous linkage is remote. Much more likely is that polarity is affected by the *rec*$^+$ genes themselves, as assumed in the following discussion.

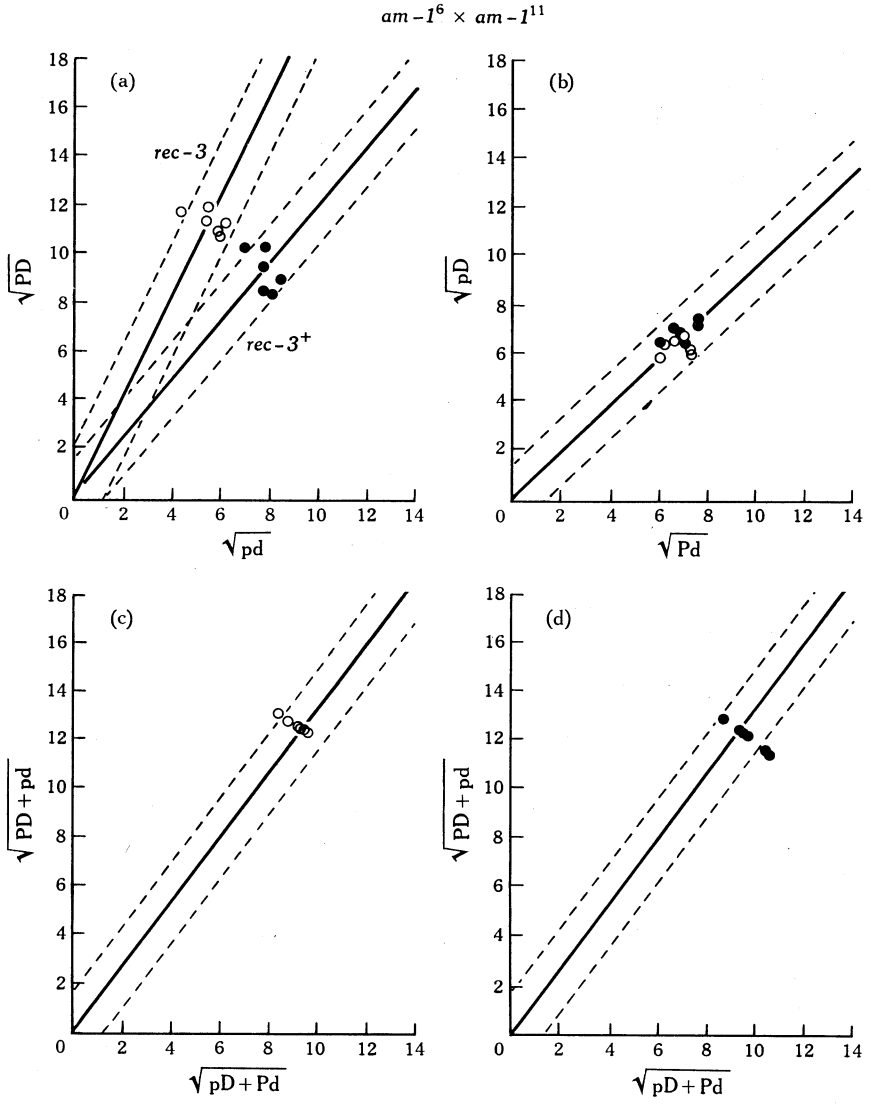


Fig. 3.—Square root charts of the distribution of flanking markers in Table 2. The means and 95% confidence limits on charts (*c*) and (*d*) are the same because they are based on the pooled results. ○ *rec-3*. ● *rec-3*$^+$.

Overall, *rec-3*$^+$ reduces *am-1*$^+$ recombinant frequency about eightfold. However, this reduction is not due to a uniform effect throughout the *am-1* locus. Prototrophic

recombinants with flanking markers PD probably result from conversion to wild type at the site of *am-1*^{6}, while those of the pd class are probably converts of *am-1*^{11}. *Rec-3*$^{+}$ increases the proportion of the latter at the expense of the former. In absolute terms, conversion at the proximal site (*am-1*^{6}) is reduced about 12-fold, while the distal site (*am-1*^{11}) shows only a fourfold reduction. Even so, more conversion probably occurs proximally in *rec-3*$^{+}$ crosses, since PD is still greater than pd.

Assuming the length and coverage of a hybrid DNA region in a locus can vary, it could include none, one, or both sites of difference in an allelic cross. Table 3 shows the possible origin of the four combinations of flanking markers among prototrophs in terms of this coverage. By relating the observed data (Table 2) to this matrix, the presumed distribution of hybrid DNA in *am-1* can be deduced.

TABLE 3

ORIGIN OF WILD-TYPE RECOMBINANTS BETWEEN ALLELIC MUTANTS m^1 AND m^2

The four classes with different combinations of flanking markers are related to different patterns of coverage of the two sites of allelic difference by hybrid DNA. The diagram on the left represents the parental chromosomes of the cross from which haploid offspring are selected. Possible formation of wild-type recombinants is shown by * in the table

Parental Chromosomes	Hybrid DNA Coverage of m^1 and m^2	Flanking Markers of + + Offspring			
		PD	pd	pD	Pd
P m^1 + D —o—|—|—|—|— —o—|—|—|—|— p + m^2 d	Proximal site	*		*	
	Distal site		*	*	
	Both sites	*	*	*	*
	Between sites			*	

Of the non-crossover classes, PD is always greater than pd—81% in *rec-3* crosses, 59% in *rec-3*$^{+}$ crosses. This is expected with preferential coverage of the more proximal site, the level of preference being reduced in the *rec-3*$^{+}$ crosses.

The relative proportions of the two classes showing crossing-over (pD and Pd) are not changed by the presence of *rec-3*$^{+}$ ($\chi^2 = 1{\cdot}190$, 1 d.f., $20\% < P < 30\%$), and are not significantly different from equality ($\chi^2 = 2{\cdot}761$, 1 d.f., $5\% < P < 10\%$). This is expected if hybrid DNA always covers both sites, assuming the differences at the sites themselves do not influence events.

Thus non-crossover and crossover classes apparently result from hybrid DNA of different extent. The former can result if hybrid DNA covers only one site, whereas the latter require both sites to be involved. The proportion of prototrophs showing crossing-over (pD+Pd/total) is not greatly affected by *rec-3*$^{+}$ ($\chi^2 = 6{\cdot}513$, 1 d.f., $1\% < P < 2\%$), and overall is only 37% of all prototrophs, so a large proportion of hybrid DNA resulting in *am-1*$^{+}$ recombinants must cover only one site.

Hybrid DNA theories explain polarity by assuming the initial breakage of single DNA chains of the two chromatids occurs *at fixed points*. The higher frequency of

recombination in the proximal region of *am-1* would result if hybrid DNA were initiated at a site proximal to the locus and if it extended for varying lengths into *am-1* before being terminated. *Rec-3*$^+$ could greatly reduce the initiation of hydrid DNA at this site. However, providing the pattern of coverage of the residual hybridity were the same, no effect on polarity would be expected.

The observed change in polarity would occur if hybrid DNA also entered *am-1* from a specific site distal to the locus at a constant level insensitive to *rec-3*$^+$. This hybridity would contribute relatively more protrophs in *rec-3*$^+$ crosses and thus increase the relative importance of recombination in the distal region. However, since more recombination occurs in the proximal region even when *rec-3*$^+$ is present (PD > pd), the proximal hybrid DNA would still predominate.

The hybrid DNA entering distally must resemble the predominant class in not being associated with crossing-over unless both sites in *am-1* are covered, and also in showing crossing-over on only about 37% of occasions when recombinants are formed. This is because *rec-3*$^+$ has no effect on either the near equality of pD and Pd or on the proportion of all recombinants of this type.

To account for the present results, the proposed sites where initiation of hybrid DNA occurs need not be outside *am-1*, they need only be proximal to the site of *am-1*^{6} and distal to that of *am-1*^{11}. However, *am-1*^{6} is the most proximal allele yet mapped and the site of *am-1*^{11} is one of the most distal known (Smyth 1970), so if the initiation sites lie in *am-1*, they must be close to the ends.

This explanation of the effect of *rec-3*$^+$ is closely similar to those which successfully account for the effect of *rec-1*$^+$ on recombination in *his-1* (Whitehouse 1966; Catcheside 1968; Thomas and Catcheside 1969). A major difference is that in *his-1* recombination the origin of the crossover classes (pD and Pd) fits the *same* pattern of coverage as the parental classes (PD and pd), whereas at *am-1* a *different* distribution of hybridity is suggested.

Initiation of hybrid DNA at a fixed point, proximal to *am-1* and sensitive to *rec-3*$^+$, implies recognition of this point by the *rec-3*$^+$ gene product or by some other product itself recognized in some way by the *rec-3*$^+$ product. This is consistent with the specificity of action of *rec-3*$^+$, which has no effect on recombination in several other loci (Catcheside 1968; Catcheside and Austin 1969). Presumably there are other recognition sites in or near these loci with specificity for other *rec* gene products.

The probable effect of *rec-3*$^+$ on the frequency of recombination in *his-2* (unlinked to *am-1*) is interesting, because it means supposed recognition sites associated with *am-1* and *his-2* must be similar or identical (Catcheside and Austin 1969 and unpublished data). It will be interesting to see if *rec-3*$^+$ has an effect on polarity of recombination in *his-2* similar to that in *am-1*.

V. Acknowledgments

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VI. References

Angel, T., Austin, B., and Catcheside, D. G. (1970).—Regulation of recombination at the *his-3* locus in *Neurospora crassa*. *Aust. J. biol. Sci.* **23**, 1229–40.

Catcheside, D. G. (1960).—Complementation among histidine mutants of *Neurospora crassa*. *Proc. R. Soc.* B **153**, 179–94.

Catcheside, D. G. (1966).—A second gene controlling allelic recombination in *Neurospora crassa*. *Aust. J. biol. Sci.* **19**, 1039–46.

Catcheside, D. G. (1968).—The control of genetic recombination in *Neurospora crassa*. In "Replication and Recombination of Genetic Material". (Eds. W. J. Peacock and R. D. Brock.) pp. 216–26. (Australian Academy of Science: Canberra.)

Catcheside, D. G., and Austin, B. (1969).—The control of allelic recombination at histidine loci in *Neurospora crassa*. *Am. J. Bot.* **56**, 685–90.

Fincham, J. R. S. (1950).—Mutant strains of *Neurospora* deficient in aminating ability. *J. biol. Chem.* **182**, 61–73.

Fincham, J. R. S. (1959).—The role of chromosomal loci in enzyme formation. Proc. 10th Int. Congr. Genet. Vol. 1. pp. 355–63.

Fogel, S., and Hurst, D. D. (1967).—Meiotic gene conversion in yeast tetrads and the theory of recombination. *Genetics, Princeton* **57**, 455–81.

Holliday, R. (1968).—Genetic recombination in fungi. In "Replication and Recombination of Genetic Material". (Eds. W. J. Peacock and R. D. Brock.) pp. 157–74. (Australian Academy of Science: Canberra.)

Jessop, A. P., and Catcheside, D. G. (1965).—Interallelic recombination at the *his-1* locus in *Neurospora crassa* and its genetic control. *Heredity, Lond.* **20**, 237–56.

Mitchell, M. B., and Mitchell, H. K. (1952).—A case of "maternal" inheritance in *Neurospora crassa*. *Proc. natn. Acad. Sci. U.S.A.* **38**, 442–9.

Murray, N. E. (1969).—Reversal of polarized recombination of alleles in *Neurospora* as a function of their position. *Genetics, Princeton* **61**, 67–77.

Pateman, J. A. (1957).—Back-mutation studies at the *am* locus in *Neurospora crassa*. *J. Genet.* **55**, 444–55.

Perkins, D. D. (1959).—New markers and multiple point linkage data in *Neurospora*. *Genetics, Princeton* **44**, 1185–208.

Smyth, D. R. (1970).—Genetic control of recombination in the *amination-1* locus of *Neurospora crassa*. Ph.D. Thesis, Australian National University.

Stadler, D. R., and Towe, A. M. (1963).—Recombination of allelic cysteine mutants in *Neurospora*. *Genetics, Princeton* **48**, 1323–44.

Thomas, P. L., and Catcheside, D. G. (1969).—Genetic control of flanking marker behaviour in an allelic cross of *Neurospora crassa*. *Can. J. Genet. Cytol.* **11**, 558–66.

Vogel, H. J. (1964).—Distribution of lysine pathways among fungi: evolutionary implications. *Am. Nat.* **98**, 435–46.

Westergaard, M., and Mitchell, H. K. (1947).—*Neurospora*. V. A synthetic medium favoring sexual reproduction. *Am. J. Bot.* **34**, 573–7.

Whitehouse, H. L. K. (1966).—An operator model of crossing-over. *Nature, Lond.* **211**, 708–13.

Whitehouse, H. L. K. (1969).—"Towards an Understanding of the Mechanism of Heredity." 2nd. Ed. [Edward Arnold (Publishers) Ltd.: London.]