CONTROL OF RECOMBINATION WITHIN THE *nitrate-2* LOCUS OF *NEUROSPORA CRASSA*: AN UNLINKED DOMINANT GENE WHICH REDUCES PROTOTROPH YIELDS

By D. E. A. CATCHESIDE*

[*Manuscript received February* 5, 1970]

Summary

The frequency of formation of prototrophic recombinants in crosses between all tested pairs of eight alleles of the *nitrate-2* locus in *N. crassa* is subject to a reduction of about eightfold by a dominant gene *rec-z*$^+$. *rec-z* is unlinked to *nit-2* and is not identical with *rec-3* or *rec-x*.

I. INTRODUCTION

A number of dominant genes which reduce the frequency of formation of prototrophic recombinants, in crosses between strains containing allelic differences, have been found in *Neurospora crassa*. *Recombination-1*$^+$ reduces the frequency of prototroph formation between *histidine-1* alleles (Jessop and Catcheside 1965; Catcheside, D. G., 1968; Catcheside and Austin 1969; Thomas and Catcheside 1969), *rec-3*$^+$ that between *amination-1* alleles (Catcheside, D. G., 1966, 1968), *rec-x*$^+$ affects *his-2* (Catcheside and Austin 1969), and *rec-4*$^+$, *rec-6*$^+$, and *rec-w*$^+$ each affect *his-3* (Jha 1967, 1969; Catcheside and Austin 1969; Angel, Austin, and Catcheside, D. G., unpublished data). Each *rec* gene is loosely linked or unlinked to the locus which it controls. *rec-1*$^+$, *rec-3*$^+$, and *rec-4*$^+$ are independent genes but it has not been rigorously shown that *rec-x*$^+$, *rec-6*$^+$, and *rec-w*$^+$ are each new loci. Indeed, it has not yet proved possible, by recombination analysis, to separate *rec-3*$^+$ and *rec-x*$^+$, which affect different loci, and they may be identical (Catcheside and Austin 1969). With this possible exception each *rec*$^+$ gene is known to affect recombination at only one locus; *rec-1*$^+$ affects only one of 10 tested loci and *rec-3*$^+$ only one of seven (Catcheside and Austin 1969; Catcheside, D. G., unpublished data).

The *rec*$^+$ gene products have been interpreted as repressor substances, either preventing access of enzymes concerned in recombination to specific loci or preventing synthesis of specific recombination enzymes. Other effects of *rec* genes have been searched for and both *rec-3*$^+$ and *rec-1*$^+$ have been found to affect the distribution of flanking markers amongst prototrophic recombinants (Catcheside, D. G., 1968; Thomas and Catcheside 1969). However, *rec* genes do not appear to affect reversion

* Research School of Biological Sciences, Australian National University, P.O. Box 4, Canberra, A.C.T. 2600.

frequency or the repressibility of gene action at the controlled loci (Catcheside, D. G., 1968; Catcheside, D. E. A., 1968*a*, 1968*b*).

Further progress in understanding the biological significance and mode of action of *rec* genes depends, to some degree, on accumulating information on the number and specificity of *rec* genes as well as finding systems open to fresh experimental approaches. New *rec* systems are, therefore, of intrinsic interest. A dominant gene, *rec-z*$^+$, which reduces the prototroph frequency in crosses between alleles of the *nitrate-2* locus is described here.

II. Materials and Methods

The approximate map locations of genes mentioned in the text are, where known, shown in Figure 1.

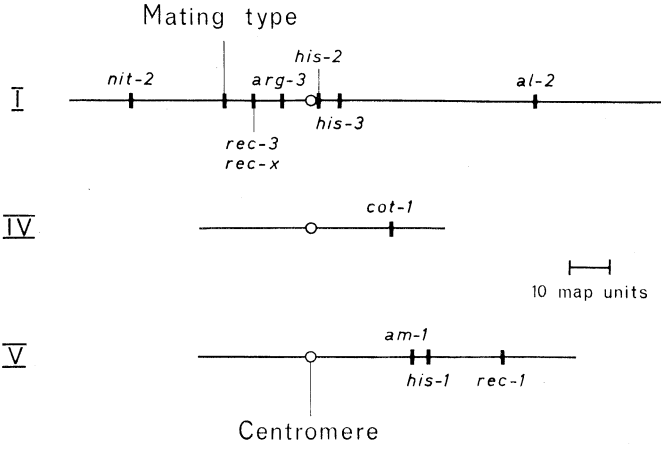

Fig. 1.—Partial linkage map of *Neurospora crassa*. The locations of *rec-4*, *rec-6*, *rec-w*, and *rec-z* are not known. (Based on Catcheside, D. G., 1968; Catcheside and Austin 1969; and Perkins *et al.* 1969.)

The stock cultures used (Table 1) were obtained from Professor D. G. Catcheside and the Fungal Genetics Stock Centre (Dartmouth College, New Hampshire). DGC3492 had been obtained from Professor J. R. S. Fincham. DGC4518 and DGC6761 were obtained by outcrossing DGC3492 for three generations, to stocks extensively bred to Emerson a and A wild types, in order to introduce *colonial temperature sensitive-1* (*cot-1*). DGC6128 was obtained by crossing the original isolate of *arginine-3* (K125), derived from Emerson a, to DGC3672, the parent of DGC6761 derived from DGC3492.

New *nit-2* alleles, MN67, MN68, MN69, MN70, MN71, MN72, and MN73, were selected, by filtration enrichment (Catcheside 1954) of ultraviolet irradiated conidia of strain 855, on the basis of their ability to prevent the leaky growth of *amination-1* mutants on Vogel's minimal medium (Catcheside, D. E. A., unpublished data). Stocks were maintained on silica-gel (Ogata 1962) and cultured on Vogel's "N" minimal medium (Vogel 1964) supplemented with sucrose 2%, 5 mM L-alanine, and solidified with 1% Ion-Agar No. 2 (Oxoid).

Crosses, incubated at 25°C, were made by mixing dense conidial suspensions in 150 by 15 mm tubes containing 4 ml of medium supplemented with 2% sucrose, 5 mM L-alanine, and a 6-cm spill folded from a 6 by 4 cm sheet of Whatman No. 1 filter paper. Although Westergaard's medium (Westergaard and Mitchell 1947) was used for some crosses, the bulk of the data was obtained from crosses which were made on media which contained 6·25 mM NH_4NO_3, in place of the KNO_3 in Westergaard's medium, as a supplement to L-alanine as a nitrogen source. This modified

medium speeded the formation of perithecia and ascospores, increased the fecundity of crosses homozygous for *nit-2*, and eliminated the problem of a high selection pressure for reversion of *nit-2* in the crossing tube.

The frequency of recombinants, prototrophic for *nit-2*, was determined by screening for the leaky growth of *nit-2*$^+$; *am-1* progeny of crosses between strains containing different *nit-2* alleles but each containing *am-1* (47305). The bulk of the spores from each crossing tube, suspended in Vogel's medium containing 2% (w/v) sucrose and 0·6% Bacto-Agar (Difco) for the 50 min heat shock at 56°C, were plated in 3-ml samples on five 20-ml plates of Vogel's medium containing 0·5% sorbose, 0·1% sucrose, and 2% Bacto-Agar. In crosses yielding very high prototroph frequencies, a 1 in 20 dilution was also plated on sorbose–sucrose medium. Of the products of the crosses, only *nit-2*$^+$ recombinants grow on this medium. The number of viable spores in the suspension was determined by plating three 3-ml samples of a 1 in 800 dilution on to plates containing 20 ml Vogel's medium supplemented with 1·0% sorbose, 0·025% glucose, 0·025% fructose, 2% Bacto-Agar, and 5 mM L-alanine. Minimal plates were incubated for 42 hr at 25°C and then transferred to 34°C for 48 hr prior to counting colonies. Alanine-supplemented plates were incubated for 18 hr at 25°C and 24 hr at 34°C. The temperature jump elicits thickening of the colonies, due to the presence of *cot-1*, aiding counting.

TABLE 1

PRIMARY CULTURES

The isolation and characterization of *nit-2* (nr37) is described by Sorger and Giles (1965). Gene symbols are defined in the text

Source Stock No.	DEAC Stock No.	Known Genetic Constitution
DGC3492	773	a *rec-3 al-2* (15300); *am-1* (47305) *rec-1*$^+$
DGC4518	855*	A *rec-3 al-2* (15300); *cot-1* (C102); *am-1* (47305)
DGC6761	1218	a *rec-3*$^+$; *cot-1* (C102); *am-1* (47305) *rec-1*$^+$
DGC6128	1742	A *rec-3 arg-3* (K125); *cot-1* (C102); *am-1* (47305) *rec-1*$^+$
FGSC♯983	2057	*nit-2* (nr37) A

* 855 is probably *rec-1*$^+$ as it was derived from a cross in which *am-1* (K314) *his-1* (K83) *rec-1* were in repulsion to *am-1* (47305) *his-1*$^+$ *rec-1*$^+$ (see Fig. 1 for linkage).

III. DISCOVERY OF *rec-z*

The new *nit-2* alleles MN67, MN68, MN69, MN70, MN71, MN72, and MN73 (stock numbers 1193–1199 respectively), obtained in strain 855, were crossed to 773 and progeny incapable of leaky growth on minimal medium (*nit-2*; *am-1*) and containing *cot-1* were selected. MN72; *cot-1*; *am-1* lines were also isolated from crosses of 1198–1218.

Crosses between various of the MN72 and MN73 isolates were found to give two distinct frequencies of prototrophic recombinants (Table 2), suggesting that the crosses 1198 × 773 and possibly 1198 × 1218 segregate for a genetic factor affecting the frequency of prototroph formation in crosses between the MN72 and MN73 alleles at the *nit-2* locus. Crosses between 773 and the original isolates of other MN alleles would also be expected to segregate for the factor since 1193–1199 have a common origin. Although the factor must be distinct from the *nit-2* locus, since it is independently inherited (Table 2), the data do not show which allele is dominant. Since 1374 yields both high and low prototroph frequency with segregants of a cross 1198 × 773, two hypotheses may be considered: either 1374, 1370, 1371, 1877, 1878, 1879, 1882, 1885, and 1887 contain the recessive allele of the factor giving high

prototroph frequency and 1883 and 1886 contain the dominant allele which reduces recombination frequency; or 1374, 1883, and 1886 contain the recessive allele giving

TABLE 2

INITIAL OBSERVATIONS OF THE TWO CLASSES OF PROTOTROPH FREQUENCY FOUND IN CROSSES BETWEEN THE *nit-2* ALLELES MN72 AND MN73

Both 1372 and 1374 were derived from a cross, 1199 × 773. Standard errors are calculated on the assumption that estimates of prototrophs and of total viable progeny conform to Poisson distributions

Source of MN72 Parent	MN72 Parent	MN73 Parent	$10^5 f$*
1198 × 1218	1693	1372	8·9±1·4
	1694	1372	8·8±1·6
	1696	1374	31·1±5·6
	1697	1374	39·6±2·5
1198 × 773	1370	1374	43·1±2·4
	1371	1374	37·2±5·8
	1877	1374	62·6±5·1
	1878	1374	47·1±4·5
	1879	1374	55·1±5·5
	1882	1374	60·5±4·9
	1883	1374	11·4±2·4
	1885	1374	56·8±4·3
	1886	1374	7·6±1·4
	1887	1374	56·3±4·9

* Frequency of prototrophic recombinants.

TABLE 3

DEMONSTRATION OF THE DOMINANCE OF LOW FREQUENCY OF PROTOTROPHIC RECOMBINANTS IN CROSSES BETWEEN *nit-2* (MN72)* AND *nit-2* (MN73)†

H = high frequency; HD = high frequency dominant; L = low frequency; LD = low frequency dominant; f = observed frequency of prototrophic recombinants

MN73 Parent	1882 (MN72 parent)			1886 (MN72 parent)		
	$10^5 f$	Expected LD	Expected HD	$10^5 f$	Expected LD	Expected HD
2202	15·2±1·9	H or L	H	6·8±1·7	L	H or L
2203	8·9±1·4	H or L	H	8·1±1·7	L	H or L
2204	8·4±1·3	H or L	H	8·5±2·0	L	H or L
2206	8·8±0·9	H or L	H	7·1±0·9	L	H or L
2207	53·8±7·3	H or L	H	6·3±0·7	L	H or L

* From 1198 × 773. † From 1199 × 773.

low frequency and 1370, 1371, 1877, 1878, 1879, 1882, 1885, and 1887 contain the dominant allele which increases recombination frequency. The consequences of these

hypotheses on the prototroph frequency classes expected from crosses of 1882 and 1886 to MN73 progeny derived from a cross 1199 × 773, which must segregate for the factor, are listed in Table 3. The results of test crosses (Table 3) are incompatible with high frequency being dominant but are entirely consistent with the hypothesis that the dominant allele of the factor reduces recombination frequency between the MN72 and MN73 alleles at the *nit-2* locus.

A compilation of all data, derived from crosses and replications of crosses between a large number of strains carrying MN72 and MN73 (Fig. 2), clearly shows the two classes of prototroph frequency. The presence of the dominant allele in both parents of a cross is correlated with a further small reduction in the prototroph yield: heterozygous $(9 \cdot 75 \pm 0 \cdot 37) \times 10^5$ ($n = 65$), homozygous $(7 \cdot 85 \pm 0 \cdot 48) \times 10^5$ ($n = 27$), ($P \simeq 0 \cdot 005$). However, this may be due to confounding by factors of small effect which are known to exist (Section VII). In either case, the factor appears to be essentially fully dominant.

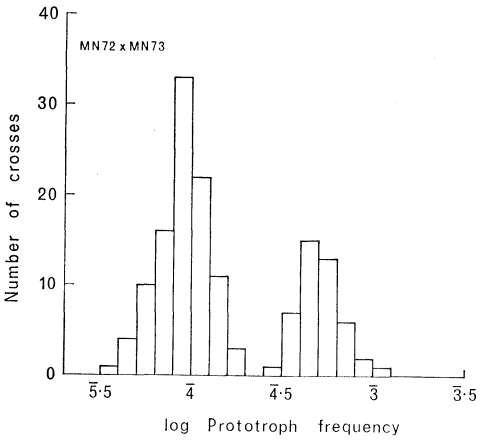

Fig. 2.—Prototroph frequencies observed in all crosses and replications of crosses between various strains carrying *nit-2* (MN72) and *nit-2* (MN73).

The major factor reducing the frequency of formation of prototrophic recombinants in crosses between this pair of *nit-2* alleles is formally similar to the *rec* genes, affecting other loci, which have been described in *N. crassa*. The dominant allele of the factor has, therefore, been temporarily denoted *rec-z*$^+$. A new locus number has not been assigned since there is no evience that *rec-z* is distinct from all of the other *rec* genes described.

1198, and hence 855, is *rec-z* (Table 4). Since 1198 × 773 segregates *rec-z*$^+$ (Table 3), 773 is *rec-z*$^+$. 1218 has also been shown to be *rec-z*$^+$.

IV. Effect of *rec-z*$^+$ on Other *nit-2* Alleles of the MN Series

As the location of *rec-z* on the genetic map of *N. crassa* is not yet known, it is not possible to follow the segregation of *rec-z* directly in crosses which do not contain either MN72 or MN73. However, crosses between segregants of the crosses between the original isolates of the new mutants and 773 (Table 4) show two levels of prototroph frequency for all pairs of alleles which have been sufficiently tested: nine pairs, MN(67 × 69, 67 × 72, 67 × 73, 70 × 71, 70 × 72, 73 × 69, 73 × 70, 73 × 71, 73 × 72), of

TABLE 4

$10^5 \times$ FREQUENCY OF PROTOTROPHIC RECOMBINANTS BETWEEN PAIRS OF *nit-2* ALLELES

Bracketed *rec-z* constitutions are deduced from the data in this table. H = high frequency, L = low frequency (data in Tables 2 and 3). With the exception of 1196 and 1198, which are the original isolates of the mutants, all stocks were derived from crosses of original mutant isolates (from 855) to 773, or, in the case of 1694, to 1218. The frequencies observed with MN70 × MN72 and MN73 × MN67 show, independently of the correlation with *rec-z*, that the *rec*$^+$ factor for recombination between each of these allele pairs is dominant in reducing recombination frequency. Standard errors are similar, in proportion, to those quoted in Tables 2 and 3

MN67		MN70			MN71	MN72	MN73			a / A		
1868 (*rec-z*)	2151 (+)	2159 (*rec-z*)	2161 (*rec-z*)	2158 (+)	2162 (+)	1694 +	1374 *rec-z*	2207 *rec-z*	2202 +			
0						15·3		285	41·5	(*rec-z*)	1867	MN67
	7·5	87·4				<2·3	4·6	4·1	3·9	(+)	1357	MN68
96·4	26·1	610				31·6	169	387	46·8	(*rec-z*)	1363	MN69
								294	45·0	(*rec-z*)	1870	MN69
						93·4		738	125	(*rec-z*)	1196	MN70
322						114		601	200	(*rec-z*)	1871	MN70
288								485	99·0	(*rec-z*)	1872	MN70
		363						190		(*rec-z*)	1874	MN71
		88·0							29·7	(+)	1875	MN71
								92·4	17·7	(*rec-z*)	1198	MN72
64·1							H			*rec-z*	1370	MN72
60·4							H	H	L	*rec-z*	1882	MN72
	9·4	567	1009	71·6	11·7		H			*rec-z*	1885	MN72
	13·2	105	105	96·1	15·0		L			+	1883	MN72
							L	L	L	+	1886	MN72
250	52·6					8·8				*rec-z*	1372	MN73
50·0	33·0					9·8	0			+	1373	MN73

the 21 possible combinations. The data of Table 4, and other data not included, are internally consistent and are explicable in terms of *rec-z*$^+$ reducing the frequency of recombination between all pairs of *nit-2* alleles; predictions of the *rec-z* constitution of a particular strain, based upon crosses with one allele, are consistent with the constitution deduced from crosses with other alleles, including MN72 and MN73 stocks of known *rec-z* constitution. If the frequency of prototroph formation between allele pairs other than MN72 and MN73 is not under the control of *rec-z* they must be controlled by other *rec* genes linked to *rec-z* since no recombinants have been observed (Table 4). The simplest hypothesis is that *rec-z*$^+$ reduces the frequency of formation of prototrophic recombinants between all tested *nit-2* allele pairs. High and low frequency of prototrophs have been observed in crosses containing each of MN67, MN69, MN70, MN71, MN72, and MN73. As the same prototroph frequency is observed in crosses between 1357 (which is MN68) and 1374, 2207, or 2202 (which are all MN73 and *rec-z*, *rec-z*, and *rec-z*$^+$ respectively) it is also probable that MN68 is subject to the effects of *rec-z*$^+$.

The contention that a single genetic factor, *rec-z*$^+$, controls the high to low change in prototroph frequency observed for all pairs of *nit-2* alleles tested, is supported by the similar multiplicative magnitude of the difference between high and low frequency for each allele pair (Fig. 3); the slope of the best fitting straight line is not significantly different from 1·0.

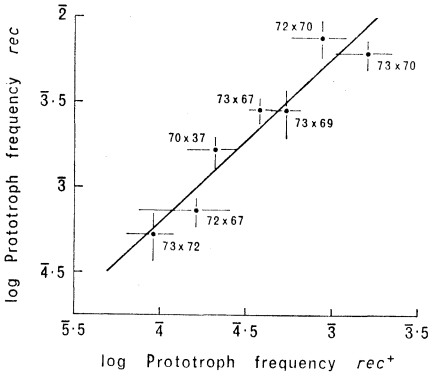

Fig. 3.—Covariation of low frequency and high frequency of prototrophic recombinants in crosses between *nit-2* alleles. The individual estimates may be affected by other genetic factors of small effect (cf. Fig. 4). The bars indicate ± one standard deviation. MN67 × MN69, MN70 × MN71, and MN73 × MN71 (Table 4) are not included as too few estimates of prototroph frequency are available. The equation of the regression is: $f_{rec} = (0{\cdot}96 \pm 0{\cdot}05) f_{rec^+} + (0{\cdot}84 \pm 0{\cdot}02)$, where f is the prototroph frequency.

V. Effect of *rec-z* on *nit-2* (nr37)

The *nit-2* mutant, nr37, which was derived prior to and independently of the MN series, has been tested for sensitivity to *rec-z*$^+$. When the *nit-2* (nr37) strain 2057 (Table 1) was crossed to 1218 (cross number 2112) and nr37 A; *cot-1*; *am-1*, progeny were isolated and crossed to the MN70 a; *rec-z* tester, 2159, only one class of prototroph frequency was observed as shown in the following tabulation:

MN70 parent	2159	2159	2159	2159	2159	2159
nr37 parent	2245	2246	2247	2248	2249	2251
$10^5 \times$ prototroph frequency	14·0±1·7	25·5±1·4	22·0±2·3	18·1±1·5	19·3±2·4	18·7±1·9

As 1218 is *rec-z*$^+$, and if *rec-z*$^+$ reduces recombination between these two alleles, it is likely that this frequency class is low frequency and that 2057 is *rec-z*$^+$ or conceivably

rec-z is linked to *am-1*. One of the nr37 a; *cot-1*; *am-1* derivatives of cross 2112 was, therefore, crossed to 855 (A; *am-1*; *cot-1*; *rec-z*) (cross 2655) and *nit-2* progeny were crossed to the MN70; *rec-z* testers, 1871 or 2159.

Two classes of prototroph frequency were observed (Fig. 4)—low frequency, similar to that found with cross 2112 progeny, and high frequency. This result is consistent with *rec-z*$^+$ reducing the frequency of prototroph formation in crosses between MN70 and nr37.

VI. Identity of *rec-z*

As 773 is *rec-z*$^+$ and 855 is *rec-z*, while both are *rec-3*, *rec-z* is not identical to *rec-3*. Twenty other independently derived MN72; *am-1* strains have been shown, directly, to be *rec-3*; *rec-z*$^+$. It is also possible that *rec-z* is not identical with *rec-1* since 855, which is *rec-z*, is probably *rec-1*$^+$ (see Table 1). A stock (1826) of constitution MN72 a *arg-3* (K125); *am-1*; *cot-1*; *rec-z*$^+$ was derived from cross 1759

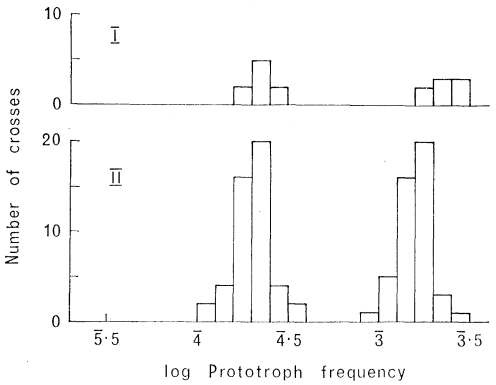

Fig. 4.—Prototroph frequencies observed in crosses between *nit-2* (MN70) and the *nit-2* (nr37) progeny of cross 2655. I, 2655 A progeny × MN70 a; *rec-z* [2159]. II, 2655 a progeny × MN70 A; *rec-z* [1871].

(1742 × 1693) and then crossed (2122) to 1885, which is MN72 A *arg-3*$^+$; *am-1*; *cot-1*; *rec-z*. Progeny of constitution A *arg-3*$^+$ were selected from cross 2122 and each was crossed to the MN73 a; *rec-z* tester, 2207. Eleven of the 20 tested were found to be *rec-z* and nine *rec-z*$^+$. Hence, *rec-z* is not located in the interval mating type to *arg-3* and cannot, therefore, be identical to *rec-x* which is located in this interval (Fig. 1). These data, which also reconfirm the non-identity of *rec-3* and *rec-z*, make it unlikely that *rec-z* is located on the left arm of linkage group I. This is confirmed by the results of cross 2655 (Fig. 4) which yielded, for *rec-z* and mating type, 56 parental and 55 recombinant combinations. Cross 2655 also segregated for *albino-2* (15300) and yielded, for *rec-z* and *al-2*, 51 parental combinations and 60 recombinants. This would make it unlikely that *rec-z*$^+$ is located on linkage group I and it is, therefore, probably unlinked and certainly not closely linked to the *nit-2* locus at which its product exerts control of recombination.

Systematic search for the map location of *rec-z*, which, when known, will permit testing of other loci for sensitivity to *rec-z*$^+$, has not yet been made. However, data acquired incidentally in the course of this investigation suggest that *rec-z* is not located in linkage group IV (18 parental: 11 recombinant with *cot-1*).

VII. Residual Variation in the Data

Contributions to peak breadth in Figure 2 and Figure 4 may be derived not only from limitations in the control of the growth environment and from sampling error, but also from minor genetic variations possibly present in the stocks used. It is noticeable that the variability present in Figure 2 is considerably greater than that in Figure 4 which is based on data derived from crosses between nr37 siblings and only two MN70; *rec-z* tester lines.

The data in Figure 2 were gleaned from several experiments involving many different MN72 and MN73 stocks, making it difficult to apportion variation between experimental and possible genetic factors. However, in Figure 4, it is apparent that crosses of the nr37 a; *rec-z* isolates to 1871 yield a different mean frequency of prototrophic recombinants from crosses of the nr37 A; *rec-z* isolates to 2159 ($P << 0{\cdot}001$). This variability can be attributed to a genetic factor either segregating in cross 2655 and linked to mating type, or differentially present in 1871 and 2159 which are themselves siblings. The small effect of this factor, 1·5-fold, would make further study difficult.

VIII. Null Effect of *rec-3* on *nit-2*

Strain 1826, and eight other MN72 a *arg-3* siblings from cross 1759 [which is homozygous *am-1* (47305); *rec-z*$^+$], were classified for *rec-3* by crossing them to an *am-1* (K314) tester (Catcheside, D. G., and Austin, unpublished data). Five were found to be *rec-3*$^+$ and four *rec-3*. In crosses of these to 1373 (which is MN73 A *rec-3*; *rec-z*$^+$), *rec-3*$^+$ was not found to reduce the yield of *nit-2*$^+$ progeny: homozygous *rec-3* $(6{\cdot}9 \pm 1{\cdot}3) \times 10^5$, heterozygous $(7{\cdot}9 \pm 1{\cdot}2) \times 10^5$. Hence, *rec-3*$^+$ does not reduce the yield of prototrophic recombinants between these *nit-2* alleles.

IX. Discussion

Like *rec-1*$^+$, *3*$^+$, *4*$^+$, *6*$^+$, *x*$^+$, and *w*$^+$, *rec-z*$^+$ is dominant in reducing the frequency of prototrophs amongst the products of recombination between allelic differences. Therefore, as in these cases it can be argued (Catcheside, D. G., 1966, 1968; Catcheside and Austin 1969) that the *rec-z*$^+$ gene product is a repressor of recombination acting either directly or indirectly on the *nit-2* locus.

Since the *rec-z*$^+$ gene is distant from its site of action, it must produce a diffusible product. The *rec-z*$^+$ product may act to reduce the formation of prototrophic recombinants between *nit-2* alleles or, alternatively, the *rec-z* product might be assumed to be active in increasing recombination frequency. However, this latter hypothesis runs into the serious problem of explaining how, in the case of this and every other *rec* gene examined, low frequency is dominant to high frequency. Such considerations make it improbable that *rec* genes specify enzymes concerned in recombination since high requency would be expected to be dominant to low frequency in all but the presumably rare cases where inactive enzyme binds more strongly to the DNA substrate. The observed degree of specificity of *rec* genes would have to be explained in terms of enzyme multiplicity if it is proposed that they specify enzymes concerned in recombination. The positive effect of *rec-1*$^+$, *rec-3*$^+$, and *rec-z*$^+$ on all tested pairs of alleles of *his-1*, *am-1*, and *nit-2* respectively makes it unlikely that their gene specificity

follows from a selective action of enzymes concerned in recombination on certain types of allelic differences.

The most attractive hypothesis is that *rec-z*$^+$ produces an active repressor which binds to a specific site in or adjacent to *nit-2*. Occupation of this site would prevent or reduce the frequency of access of recombinational enzymes to the *nit-2* gene and perhaps also adjacent regions of linkage group I. Alternatively, the *rec-z*$^+$ gene product might be the repressor for a gene specifying an enzyme concerned in recombination which is specific to *nit-2* and perhaps a restricted number of other loci.

Each of these hypotheses predicts that there must be specific sites within or adjacent to the gene at which recombination is controlled, analogous to an operator in the former case and a promoter in the latter case. Specificity of one *rec* gene for more than one locus, such as that which may exist for *his-2* and *am-1*, would, in both hypotheses, be achieved by similar recognition sites associated with each gene. A genetic factor for *his-3*, with the properties predicted for such a recognition site, has been found (Angel, Austin, and Catcheside, D. G., unpublished data).

The examination, in *N. crassa*, of the effect of *rec* genes on recombination between allelic differences, has been restricted to measuring the frequency of prototrophic recombinants and the distribution of flanking markers amongst them. Of the possible types of recombination product, only the one which can be selectively isolated has been examined, due to the low frequency of recombination events in the systems examined so far. Since very high prototroph frequencies are attained (up to 0·01) the *nit-2*; *rec-z*$^+$ system offers the possibility of studying the effects of *rec* genes by the technique of tetrad analysis, in which all products of one recombination event are observed. Use of this technique may resolve some of the ambiguities which render interpretation of the mode of action of *rec* genes difficult and, by adding a further but controlled variable, may amplify the resolution of tetrad analysis upon the mechanism of recombination.

X. Acknowledgments

I should like to thank Professor D. G. Catcheside for providing stock cultures of *N. crassa*, for determining the *rec-3* constitution of some of the *nit-2*; *am-1* strains derived during this investigation, and for permission to quote his unpublished observations. I am grateful to Miss D. Combes and Mr. N. Gowen for their excellent technical assistance.

XI. References

Catcheside, D. E. A. (1968*a*).—The mechanism of genetic regulation of recombination and gene expression in *Neurospora crassa*. In "Replication and Recombination of Genetic Material". (Eds. W. J. Peacock and R. D. Brock.) pp. 227–8. (Australian Academy of Science: Canberra.)

Catcheside, D. E. A. (1968*b*).—Regulation of the *am-1* locus in *Neurospora*: evidence of independent control of allelic recombination and gene expression. *Genetics, Princeton* **59**, 443–52.

Catcheside, D. G. (1954).—Isolation of nutritional mutants of *Neurospora crassa* by filtration enrichment. *J. gen. Microbiol.* **11**, 34–6.

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**(7), 685–90.

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.

JHA, K. K. (1967).—Genetic control of allelic recombination at the *histidine-3* locus of *Neurospora crassa*. *Genetics, Princeton* **57**, 865–73.

JHA, K. K. (1969).—Genetic factors affecting allelic recombination at the *histidine-3* locus of *Neurospora crassa*. *Molec. Gen. Genetics* **105**, 30–7.

OGATA, W. N. (1962).—Preservation of *Neurospora* stock cultures with anhydrous silica gel. *Neurospora Newsl.* **1**, 13.

PERKINS, D. D., NEWMEYER, D., TAYLOR, C. W., and BENNETT, D. C. (1969).—New markers and map sequences in *Neurospora crassa*, with a description of mapping by duplication coverage, and of multiple translocation stocks for testing linkage. *Genetica* **40**, 247–78.

SORGER, G. J., and GILES, N. H. (1965).—Genetic control of nitrate reductase in *Neurospora crassa*. *Genetics, Princeton* **52**, 777–88.

THOMAS, P. L., and CATCHESIDE, D. G. (1969).—Genetic controls 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 favouring sexual reproduction. *Am. J. Bot.* **34**, 573–7.