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A Second Locus Subject to Recombination Control by the *rec-1*$^+$ Gene in *Neurospora crassa*

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Abstract

The yield of prototrophic recombinants in crosses between *nit-2* alleles is repressed in the presence of the *rec-z*$^+$ gene. Similarly, recombination between *his-1* alleles is repressed by the *rec-1*$^+$ gene. Experiments reported in this paper show that if the *rec-1*$^+$ and *rec-z*$^+$ loci are different, the worst estimate indicates that they are located not more than 0·67 map units apart with 95% probability. It is likely, therefore, that *rec-1* controls the frequency of recombination between *nit-2* alleles as well as between *his-1* alleles. This is in line with evidence that each of the genes *rec-2*$^+$ and *rec-3*$^+$ repress recombination in more than one chromosomal segment. Nevertheless, each *rec* gene is specific in its action, repressing recombination in only a limited fraction of the genome.

Introduction

Three distinct genes that are dominant in reducing recombination in specific regions of the genome have been described in *Neurospora crassa*. The gene *rec-1*$^+$* was first recognized for its effect on recombination between *his-1* alleles (Jessop and D. G. Catcheside 1965), *rec-2*$^+$ for its effect on recombination between the *pyr-3* and *his-5* loci (Smith 1966) and *rec-3*$^+$ for its effect on recombination between *am-1* alleles (D. G. Catcheside 1966).

Subsequently a number of other cases in which reduction of recombination could be attributed to a single dominant gene were found: *rec-x*$^+$ affecting *his-2* alleles (D. G. Catcheside and Austin 1969), *rec-4*$^+$ and *rec-w*$^+$ each affecting *his-3* alleles (Jha 1967; D. G. Catcheside and Austin 1969) and *rec-z*$^+$ affecting recombination between *nit-2* alleles (D. E. A. Catcheside 1970).

Further investigation has shown that *rec-x* fails to recombine with *rec-3* (D. G. Catcheside and Austin 1971), that *rec-w* does not recombine with *rec-2* and that it is unlikely that *rec-4* is not *rec-2* (D. G. Catcheside and Corcoran 1973). The simplest interpretation of these results is that the specificities of *rec-2* and *rec-3* are not confined to single chromosome segments but that each controls recombination in at least two regions, *rec-x* being a synonym for *rec-3* and *rec-w* and *rec-4* being synonyms for *rec-2*.

In addition, recombination in a number of segments in linkage group I has been found to be reduced by dominant genes having map locations that could not be

* The following abbreviations are used in this paper: *rec-1, recombination-1; ad-3, adenine-3; al-2, albino-2; am-1, amination-1; arg-3, arginine-3; asp, asparagine; cot-1, colonial temperature sensitive-1; his-1, histidine-1; inos, inositol; lys-5, lysine-5; nic-3, nicotinamide-3; nit-2, nitrate-2; nt, nicotinamide–tryptophan; pan-2, pantothenic acid-2; pyr-3, pyrimidine-3; ro-4, ropy-4; sn, snowflake; tryp-2, tryptophan-2; ylo-1, yellow-1.*

separated from that of either *rec-2* or *rec-3* (D. G. Catcheside and Corcoran 1973). Thus, *rec-2*$^+$ is now implicated in reducing the frequency of recombination between *his-3* alleles (I)* and in the chromosome segments *pyr-3–his-5* (IV), *arg-3–sn* (I) and *his-3–ad-3* (I), and *rec-3*$^+$ is implicated in reducing recombination between *am-1* alleles (V), between *his-2* alleles (I) and in the chromosome segment *sn–his-2* (I).

These data do not indicate that each *rec*$^+$ gene influences recombination throughout the genome. The *rec-3*$^+$ gene is known to have no effect upon recombination in 12 chromosomal regions. Likewise, *rec-2*$^+$ has no effect upon 13 regions and *rec-1*$^+$ no effect upon 19 different chromosomal segments. Furthermore, in each of the chromosome segments where recombination is known to be controlled, only one of the three *rec*$^+$ genes is effective; the other two have no detectable effect (D. G. Catcheside and Austin 1969; D. G. Catcheside, personal communication).

In this paper, data which indicate that *rec-1*$^+$ reduces recombination between *nit-2* alleles (I) as well as between *his-1* alleles (V) is presented. This follows from the finding that *rec-z*$^+$, defined for its effect upon recombination between *nit-2* alleles, does not recombine with *rec-1*$^+$. Hence, each of the three known *rec*$^+$ genes in *N. crassa* are alike in that they control recombination at more than one chromosomal location.

Table 1. Genotype and derivation of cultures

Stock number	Source[A]	Known genotype[B]
855	1	*A rec-3 al-2; cot-1 am-1 rec-z*
1218	1	*a rec-3*$^+$*; cot-1; am-1 rec-1*$^+$ *rec-z*$^+$
1374	1	*nit-2* (MN73) *a rec-3 al-2; cot-1; am-1 rec-z*
1693	1	*nit-2* (MN72) *a rec-3*$^+$ *al-2; cot-1; am-1 rec-z*$^+$
1728	7	*nit-2* (MN72) *A rec-3 al-2; am-1 ad-7* (MN227) *rec-z*$^+$
1885	1	*nit-2* (MN72) *A rec-3 al-2; cot-1; am-1 rec-z*
1886	1	*nit-2* (MN72) *A rec-3 al-2; cot-1; am-1 rec-z*$^+$
2207	1	*nit-2* (MN73) *a rec-3 al-2; cot-1; am-1 rec-z*
2794	2	*a al-2; cot-1; am-1 his-1* (K83) *rec-1*
3869	3	*A; cot-1; his-1* (K651) *inos rec-1*
3871	4	*A; cot-1 inos asp* (C123) *rec-1*$^+$
3872	5	*a; cot-1; am-1*^{6} *his-1* (K83) *ad-7* (K77) *rec-1*$^+$
4212	7	*nit-2* (MN72) *a al-2; cot-1; am-1 inos his-1* (K83) *asp* (C123) *rec-1*$^+$ *rec-z*$^+$
4277	7	*nit-2* (MN72) *A; cot-1; am-1 his-1* (K83) *ad-7* (K77) *rec-1*$^+$ *rec-z*$^+$
4322	6	*a; cot-1; his-1* (K625) *inos rec-1*
4801	7	*a al-2; cot-1; am-1 his-1* (K83) *rec-1 rec-z asp* (MN137)

[A] Sources of stocks were as follows: *1*, origin or derivation described by D. E. A. Catcheside (1970); *2, 3, 4, 5* and *6*, D. G. Catcheside stock numbers 4208, 3662, 7116, 8939 and 6214 respectively; *7*, construction described in the text.

[B] In all cases the *al-2* allele is 15300, the *cot-1* allele is C102, the *inos* allele is 37401 and, with the exception of stock 3872, which contains *am-1*^{6}, the *am-1* allele is 47305.

Materials and Methods

Cultures

The following stocks, used for testing the linkage of *rec-z* to group II, III, VI and VII markers, were obtained from D. G. Catcheside: II *a arg-5* (27947) and *a tryp-3* (A78) : III *A ad-2* (K170) *tryp-1*

* Roman numerals refer to linkage groups.

(10575) : VI *a lys-5* (DS6-85) *ylo-1* (Y30539y) and *a tryp-2* (A60) : VII *a nt* (65001). The group VII marker stock: *A nic-3* (Y31881) was obtained from the Fungal Genetics Stock Centre (FGSC stock number 121).

The genotype and origin of all other cultures is detailed in Table 1.

Stock number 1728 was obtained by crossing 1693 to D. G. Catcheside's stock number 3814, an adenine-requiring isolate (*A his*$^+$ *crisp*$^+$) derived from a cross between Emerson *A* wild type and FGSC stock number 246, which is *a ad-5* (Y152 M40) *his-2* (Y152 M14) *crisp* (B123). Analysis showed DGC 3814 to contain a new *ad-7* allele rather than *ad-5*. The spontaneous acquisition of additional mutations blocking the adenine pathway in adenine auxotrophs is a well-known phenomenon (Mitchell and Mitchell 1950) and presumably this event occurred in FGSC 246. Unlike the *ad-5* allele, the new mutant (MN227) is not linked to *his-2* and *crisp* (see Table 2) and hence would be expected to be the predominant *ad* allele amongst *his*$^+$ *crisp*$^+$ progeny obtained when FGSC 246 is crossed to wild type. The location of *ad* (MN227) 14·7 map units from *am-1* on linkage group V suggested that it is an *ad-7* allele. This hypothesis was tested by constructing a forced heterokaryon with the following genotype: [*a al* (15300); *ad* (MN227) *asp* (MN137)] + [*nit-2* (MN72) *a*; *cot-1* (C102); *am-1*^{6} *his-1* (K83) *ad-7* (K77)]. Since the heterokaryon fails to grow unless adenine is present in the culture medium, the *ad-7* (K77) and *ad* (MN227) mutations are allelic; they affect the same function in adenine biosynthesis.

Table 2. Linkage data for *ad* (MN227) obtained from a cross between DGC 3814 and 1693

The data are abstracted from determinations of the full genotype of 190 viable ascospores

Gene pair[A]	Total number of: Parental types	Recombinant types	P[B]	Map distance (centimorgans)
ad nit-2	96	94	0·9	—
ad mating type	102	88	0·3	—
ad al-2	104	86	0·2	—
ad am-1	162	28	$\leqslant$0·001	14·7

[A] The markers *nit-2*, *al-2* and mating type are located on linkage group I and *am-1* on linkage group V. Since *ad* (MN227) is linked to *am-1* it will not show linkage to the group I markers *his-2* and *crisp* present in FGSC 246.

[B] Probability of obtaining as great or greater deviation from equality of parental and recombinant types if the genes are truly independently inherited.

Stock 4212 was isolated from a cross between 3882 [an isolate from a cross between 3871 and 2794 having the genotype *A al-2; cot-1; am-1 his-1 inos asp* (C123) *rec-1*$^+$] and 1880 [a sibling of 1885 having the genotype *nit-2* (MN72) *a rec-3 al-2; cot-1; am-1 rec-z*].

Stock number 4277 was constructed as follows: from a cross between 1885 and 3872 a strain having the genotype *nit-2* (MN72) *A al-2; cot-1; am-1*^{6} *his-1 ad-7 rec-1*$^+$ was isolated. The *rec* constitution was determined in crosses to the testers 4322 and 2207. 4277 proved to contain both *rec-1*$^+$ and *rec-z*$^+$ (indicating that *rec-z*$^+$ was present in 3872). The *am-1* (47305) allele present in 4277 was identified by examining the properties of the NADP-specific glutamate dehydrogenase in cell-free extracts, using the methods detailed by D. E. A. Catcheside (1968). The mutant enzyme from *am-1* (47305) cells retains the ability to deaminate glutamate whilst the enzyme from *am-1*^{6} cells is unable either to deaminate glutamate or to aminate α-ketoglutarate.

Stock 4801 was constructed in two steps. First, a new mutation causing a requirement for asparagine, *asp* (MN137), was inserted into stock 2794 by mutation. This was achieved by treating conidia with ultraviolet light followed by filtration enrichment using the method of D. G. Catcheside (1954). Next, the new mutant was crossed to 1883 (a sibling of 1886 having the same genotype). Determination of the genotypes of a random sample of ascospores revealed that 21 were *asp his* and 25

were + + parental types, while 4 were + *his-1* and 4 were *asp* + recombinant types. These results indicate that *asp* (MN137) is linked to *his-1* on linkage group V. Three-point mapping has shown *asp* (MN137) to be distal to *ad-7* (D. E. A. Catcheside 1973) and thus close to the location of the *asp* (C123) gene (D. G. Catcheside and Austin 1969). A forced heterokaryon was constructed, having the genotype: [*nit-2* (MN72) *a*; *am-1* (47305) *his-1* (K83) *inos* (37401) *asp* (C123)] + [*nit-2* (MN72) *a*; *am-1* (47305) *his-1* (K83) *ad-7* (K77) *asp* (MN137)]. The heterokaryon grows on media supplemented with alanine, histidine and asparagine but growth stops following transfer to media supplemented only with alanine and histidine. Hence, the *asp* (C123) and *asp* (MN137) mutations are allelic; they block the same function in asparagine synthesis. Stock 4801 was selected from amongst the *his-1 asp* parental-type progeny of the cross between the original isolate and 1883. The presence of *rec-1* was ascertained in crosses to the tester 3869 and the presence of *rec-z* was confirmed by the detection of *rec-z* segregants amongst the progeny of cross 4805 (see Results section). A direct breeding approach to obtaining the genotype of 4801 was avoided because of the close linkage of *asp* to the *rec-1* locus, the lack of this gene combination in stock cultures and the consequent large investment of effort in *rec-1* tests that would be required.

Cultures were preserved on silica gel (Ogata 1962) to minimize the problem of genetic selection and drift during storage.

Media, Crossing Methods and Recombination Assays

These were as described in D. E. A. Catcheside (1970), with the following additions. Where needed, supplements were added to media in the following concentrations: adenosine 0·8 mM, adenine 0·8 mM, arginine 2·9 mM, asparagine 1·5 mM, histidine 1·3 mM, inositol 100 μM, lysine 2·7 mM, nicotinamide 30 μM, tryptophan 2·0 mM and uracil 0·9 mM. The method of determining the frequency of prototrophic recombinants between *his-1* alleles in *rec-1* test crosses was similar to that described for *nit-2* alleles (D. E. A. Catcheside 1970) except that the selective plates contained sorbose–glucose–fructose medium and were incubated for 18 h at 25°C prior to incubation at 34°C for 24 h, and histidine was used in place of alanine in non-selective plates.

Determination of Genotypes

The *rec* constitution of cultures was determined by measuring, for the appropriate locus, the frequency of prototrophic recombinants obtained in crosses between the unknown and a tester containing the recessive allele of the *rec* gene. Hence, the unknown and tester must contain different alleles of the appropriate test locus, *nit-2* for *rec-z*/*z*$^+$ tests and *his-1* for *rec-1*/*1*$^+$ tests. Since the dominant allele reduces recombination, the prototroph yield is lowered (by about 10-fold) if the unknown is *rec*$^+$.

Auxotrophic mutations were detected by testing the ability of conidia to germinate and grow on medium deficient in the specific supplement, and mating type was determined in crosses to the '*fluffy*' mating type testers (Perkins *et al.* 1962). *nit-2* Mutants are normally scored by their ability to prevent the adaptation of *am-1* to grow on minimal medium. Presumably this is due to prevention of the derepression of NAD-dependent glutamate dehydrogenase which can take over the function of the NADP-dependent glutamate dehydrogenase that is lacking in *am-1* cells. Subtle alteration of the morphology of growth, a thinner and somewhat delicate habit, was found to be a reliable guide to scoring *nit-2* when the presence of mutations requiring supplements containing amino groups prevents the usual auxanographic method. In critical cases this was checked by testing the progeny of crosses to suitable *nit-2*$^+$ stocks for the presence of the *nit-2* gene.

Linkage Tests for the rec-z *Gene*

Tests for linkage to group II, III, VI and VII markers (*arg-5 tryp-3; ad-2; lys-5 ylo-1 tryp-2; nic-3 nt*) were instigated by breeding *am-1* and *cot-1* (by crosses to 855 or 1218) into stocks containing one or more of the markers. The resultant progeny were used for mapping; they were crossed to *rec-z* and *rec-z*$^+$ stocks of the appropriate mating type which also contained *nit-2* (MN73) as well as *am-1* (47305) and *cot-1* (C102). It was necessary to use stocks of both *rec-z* and *rec-z*$^+$ genotype to be certain of obtaining a cross segregating for the *rec* gene, since there was no way of ascertaining the *rec* constitution of the original marker stocks. From each of these mapping crosses about 50 progeny that were wild type with respect to the segregating nutritional marker and that contained *nit-2* (MN73) were selected. Each of the selections was then crossed to a *rec-z* tester of the appropriate mating type

having the constitution *nit-2* (MN72); *cot-1* (C102); *am-1* (47305), *rec-z*. Thus for each of the eight nutritional markers there were two sets of test crosses: one set was expected to show only the high or the low frequency of prototrophic recombinants typical of crosses between *nit-2* (MN72) and *nit-2* (MN73), reflecting the fact that the *rec* gene was not segregating in the mapping cross, and in the second set *rec-z* and *rec-z*$^+$ were expected to segregate in the mapping cross and therefore both high recombination frequencies typical of *rec-z* × *rec-z* crosses and low frequencies typical of *rec-z*$^+$ × *rec-z* crosses were expected to be found. The linkage test would be completed by determining which sets of crosses were segregating for high and low recombination frequencies and whether or not the ratio of high : low differed significantly from unity.

Results

Assignment of rec-z$^+$ *to a Linkage Group*

Previous work had shown that *rec-z* is not linked to mating type, *al-2* or *cot-1* and hence is unlikely to be located on either of linkage groups I or IV (D. E. A. Catcheside 1970). Tests for linkage to group II, III, VI and VII markers (see Methods) were discontinued when linkage to group V markers was detected.

Tests for linkage to group V markers were facilitated by the availability of stocks with a known *rec-z* constitution and were stimulated by the known presence of the *rec-1* and *rec-2* loci on linkage group V. With the available stocks the most rapid tests for linkage to group V markers were the crosses detailed in Fig. 1 (cross numbers 2870 and 2872).

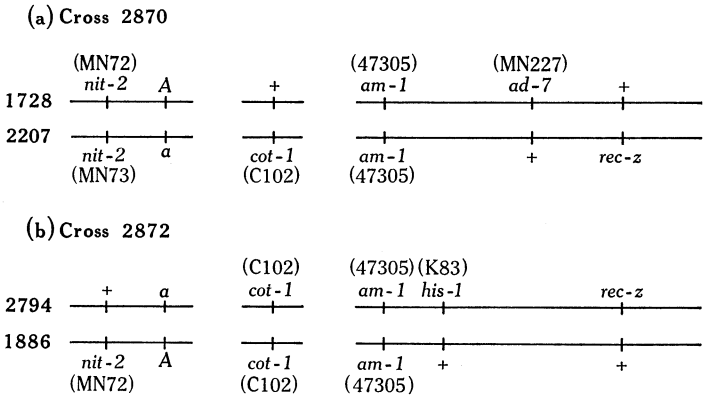

Fig. 1. Partial genotypes of the mapping crosses that locate *rec-z* on linkage group V. Map distances are not to scale.

(i) *Linkage to* ad-7

In addition to being heterozygous for *rec-z* and for the group V marker *ad-7*, cross 2870 also segregated the group IV marker *cot-1* and the group I markers *nit-2* and mating type. Segregants having the genotype *cot-1 ad-7*$^+$ were selected, the mating type was determined and then the segregants were crossed to *rec-z* testers of the appropriate mating type, either stock number 2207: *nit-2* (MN73) *a*, or stock number 1885: *nit-2* (MN72) *A*.

Four of the 42 isolates tested for their *rec* constitution were recombinant between mating type and *nit-2*. In these cases the test crosses were homozygous for one of the *nit-2* alleles and consequently produced no *nit-2*$^+$ recombinants. Of the remaining

isolates 22 were *A* and 16 *a*, and all 38 tested as *rec-z*, giving the high frequency of prototrophic recombinants expected of *rec-z* × *rec-z* crosses (Fig. 2*a*).

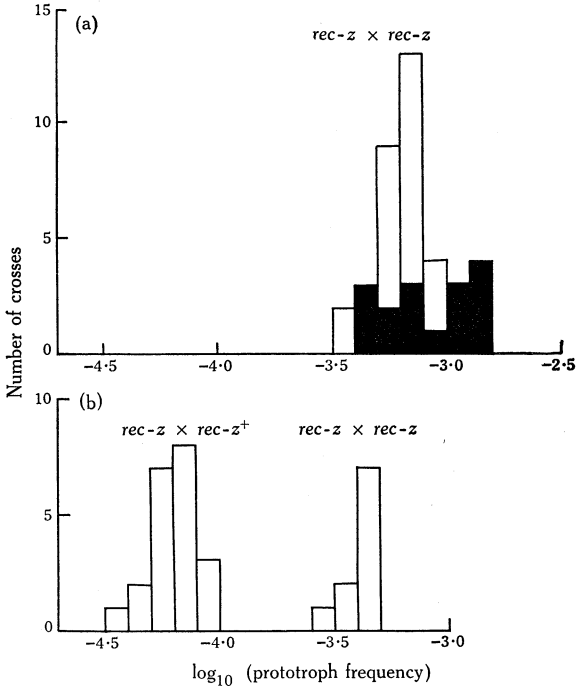

Fig. 2. Frequencies of *nit-2*$^+$ prototrophs obtained in *rec-z* tests. (*a*) *cot-1; ad-7*$^+$ isolates from cross 2870 mated with the *rec-z* testers 2207 (open histogram) or 1885 (solid histogram). The results indicate that all of the isolates of 2870 scored are *rec-z*$^+$; nevertheless, there is evidence of another factor affecting the yield of *nit-2*$^+$ recombinants (see text). (*b*) *nit-2 A*; *his-1*$^+$ isolates from cross 2872 mated to the *rec-z* tester 2207.

The results are consistent with *rec-z* being located less than 7·6 map units (95% confidence limit) from either *cot-1* or *ad-7*. Since previous experiments had indicated that there is no linkage between *rec-z* and *cot-1* (D. E. A. Catcheside 1970), this result is consistent only with *rec-z* being linked to *ad-7* which is located on linkage group V. The presence of *rec-z*$^+$ in 2870 was confirmed by selecting four progeny with the genotype *nit-2* (MN72) *A*; *cot-1; ad-7* (MN227) and crossing them to the *rec-z* tester, 2207. All four gave the low prototroph frequencies expected of *rec-z* × *rec-z*$^+$ test crosses.

(ii) *Linkage to* his-1

Thirty-one progeny from cross 2872 (Fig. 1*b*) with the genotype: *nit-2 A*; *his-1*$^+$ were selected and crossed to 2207 to test their *rec-z* constitution. In all, 21 progeny were found to be parental type (*rec-z*$^+$) and 10 were recombinant (*rec-z*) (Fig. 2*b*). Thus the frequency of recombination between *his-1* and *rec-z* in cross 2872, expressed as a percentage, is 32·3±8·4, a result that would be obtained with a probability of less than 0·05 if *his-1* and *rec-z* were not linked.

Both mapping crosses concur in indicating linkage of *rec-z* to group V markers, the most probable location of *rec-z* being distal to *his-1*, close to *ad-7*. This is close to the known location of *rec-1*, 21·0±2·9 map units distal from *his-1* and between *ad-7* and *asp* (D. G. Catcheside and Austin 1969), but is not close to the location of *rec-2*, proximal to *his-1* and adjacent to *am-1* (D. G. Catcheside and Corcoran 1973).

These results reinforced the suspicion that *rec-z*$^+$ might be identical with a previously described *rec* gene, and more specifically, that *rec-1*$^+$ affects recombination frequencies not only between alleles of the *his-1* locus, but also between *nit-2* alleles, even though *nit-2* is located on a different linkage group.

(iii) *Anomalous prototroph frequencies in* rec-z *tests*

The prototroph frequency distribution in the *rec-z* tests plotted in Fig. 2*a* is somewhat higher and more variable than that typical of crosses homozygous for *rec-z* (cf. Fig. 2*b*). In addition, if the data for the crosses to the 1885 tester are examined separately there is evidence of a bimodal distribution. This is consistent with the segregation of a genetic factor (other than *rec-z*) that has a minor effect on the yield of *nit-2*$^+$ recombinants. A factor with a similar small effect upon recombination between *nit-2* alleles has already been detected (D. E A. Catcheside 1970). Analysis of the pedigrees of the stocks involved in the current and the 1970 experiments shows that a single gene could be responsible for both sets of anomalous recombination data. As a guide to further investigation of this phenomenon, it could be inferred that 2207 contains a dominant allele that determines low frequency, that 1885 and 1728 contain the recessive allele and that the locus is unlikely to be located on linkage group IV, V or the left arm of linkage group I. However, systematic pursuit of this gene would be difficult in view of its rather small effect upon recombination.

Testing rec-z *and* rec-1 *for Identity*

The recessive alleles of the *rec-z*$^+$ and *rec-1*$^+$ genes were discovered in different laboratory stocks, not by mutant induction and isolation. Indeed, no practicable way of selecting and isolating induced genetic variants of *rec* genes is available as yet. Consequently, deliberate mutation cannot be used at present as a method of testing *rec-1* and *rec-z* for identity.

The only available method is that of recombination analysis: analysis of the progeny of a cross for recombination between *rec-1* and *rec-z*. The efficiency of this method was increased by bracketing the chromosome segment containing the *rec* genes with the closest known proximal marker (*ad-7*) and the closest known distal marker (*asp*) and restricting attention to progeny in which these flanking markers were recombined. Since this reduces the number of *rec* tests needed, a moderate resolution test for recombination between *rec-1* and *rec-z* is feasible.

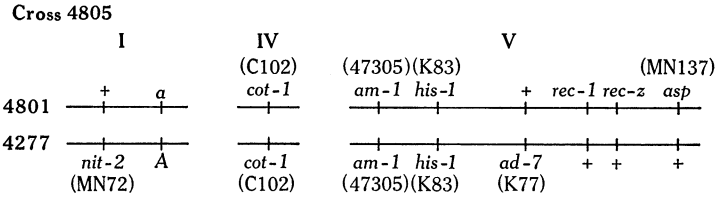

Fig. 3. Partial genotype of cross 4805 from which recombinants between *rec-1* and *rec-z* were sought. Map distances are not to scale.

In addition to being heterozygous for each of *rec-1*, *rec-z*, *ad-7* and *asp*, the test cross must be homozygous for *his-1* and *am-1* and must also contain *nit-2* to permit

determination of the *rec-1* and *rec-z* constitution of the progeny. The technical requirements of the *rec* tests also demand the presence of *cot-1* in both parents. The parental strains used in cross 4805 (Fig. 3) meet these criteria. Construction of these stocks is described in the Methods section.

(i) *Frequency of recombination between* ad-7 *and* asp

Random spore analysis of cross 4805 for recombination between *ad-7* and *asp* yielded the following results:

Parental types		Recombinant types		Total	Percentage germination
ad-7 +	58	*ad-7 asp*	9	145	56·6
+ asp	66	+ +	12		

The somewhat poor germination is not reflected in a gross distortion of segregation in the *ad-7–asp* region, nor is there any indication of chromosomal aberration in the stocks. The minor loss of progeny containing the *ad-7* gene was highly significant in a sample taken earlier, shortly after ascospore discharge, and poor germination in this cross may reflect a number of such metabolic difficulties resulting from the presence of a multiplicity of auxotrophic mutations in the parental strains.

In order to obtain a better estimate of the map distance between *ad-7* and *asp*, a sample of ascospore suspension from cross 4805 was plated on selective medium (minimal plus histidine and alanine) and an equal volume of a 10-fold dilution plated on fully supplemented medium (minimal plus histidine, alanine, asparagine and adenosine). A total of 359 colonies grew on selective medium and 376 colonies on fully supplemented medium. Pooling the data from both samples of cross 4805 progeny yields a best estimate for the distance between the *ad-7* and *asp* loci of $18{\cdot}4 \pm 1{\cdot}2$ map units. This contrasts sharply with some other estimates of the *ad-7–asp* interval: $9{\cdot}5 \pm 3{\cdot}7$ in cross 5258 (D. E. A. Catcheside 1973) and $9{\cdot}02 \pm 0{\cdot}26$ in a cross (number 4369) between 4277 and 4212. The pedigrees of the stocks involved are consistent with this twofold variation in recombination frequency being due to a dominant repressor gene.

(ii) *Analysis of cross 4805 for recombination between* rec-1 *and* rec-z

Recombinants with the constitution *ad-7*$^+$ *asp*$^+$ were selected by plating ascospores on minimal medium supplemented only with histidine and alanine. These were then tested for mating type and for *nit-2*. In all, 82 segregants with the genotype *nit-2* (MN72) *A*; *cot-1* (C102); *am-1* (47305) *his-1* (K83) *ad-7*$^+$ *asp*$^+$ were isolated. Each of these were crossed to *rec-1* and *rec-z* testers (4322 and 1374 respectively). Since the recombination frequency between *ad-7* and *asp* flankers is $18{\cdot}4\%$ in cross 4805, screening 82 *ad-7*$^+$ *asp*$^+$ recombinants for recombination between *rec-1* and *rec-z* is equivalent to screening 446 randomly selected progeny from cross 4805 for this event.

In Fig. 4 the frequency of *his*$^+$ recombinants obtained in the *rec-1* test cross is plotted against the frequency of *nit*$^+$ recombinants obtained in the *rec-z* test. The parental types cluster in the lower left (*rec-1*$^+$, *rec-z*$^+$) or in the upper right (*rec-1 rec-z*). Progeny in which *rec-1* and *rec-z* have recombined are expected to group in one or other of the remaining corners of the plot, i.e. the upper left and lower right.

Although the bulk of the *ad-7*$^+$ *asp*$^+$ progeny are clearly of parental type with respect to the two *rec* genes, isolate numbers 198, 208, 231 and 296 appear to be *rec-z rec-1*$^+$ recombinants. However, this result could also be obtained if a reversion of *nit-2* occurred in the crossing tube giving rise to one or more +/*nit-2* perithecia. To check this possibility the *rec-z* determinations were repeated. The following frequencies of *nit*$^+$ recombinants per 10^5 viable ascospores were observed:

	Isolation number:			
	198	208	231	296
Initial cross	39·7±11·5	44·4±4·6	53·9±9·8	106±7·4
Repeat crosses	13·9±3·8	11·7±2·7	14·2±3·2	12·7±2·2
		10·8±2·4		11·8±1·9

In each case the isolates proved to be *rec-z*$^+$ and therefore not *rec-1*$^+$ *rec-z* recombinants.

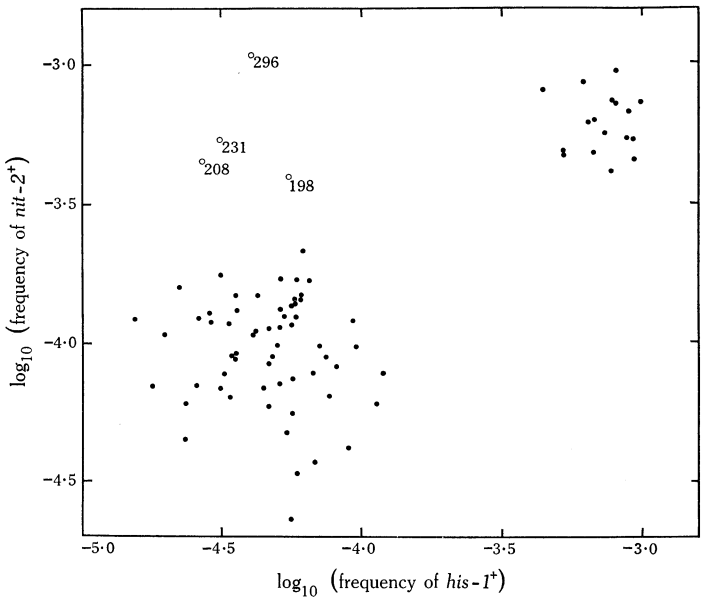

Fig. 4. Frequency of recombination observed in the first *rec-1* and *rec-z* tests made upon the 82 *ad-7*$^+$ *asp*$^+$ recombinants from cross 4805. The frequency obtained in the *rec-1* test cross to 4322 is plotted against the frequency obtained in the *rec-z* test cross to 1374. The results are equivocal in some cases (see text).

The grouping of probable *rec-1*$^+$ *rec-z*$^+$ parental types (Fig. 4) is not compact, making the *rec* genotype uncertain for some of the segregants. The scatter is a result of the relatively small number of prototrophic recombinants obtained in some of these crosses, reflecting an error in the estimate rather than an intrinsic large variability in the recombination frequency. In order to overcome this imprecision, *rec* determinations for isolates plotting at the periphery of the group were repeated by setting up and analysing one or more further crosses. The results of the repeat determinations were *added* to the first determination. As a result, the number of prototrophic

recombinants observed in these repeated crosses was raised to a mean of 34 with a median number of 23 and an absolute minimum of 11 in the case of the most intractably infertile isolate.

The best available estimates for recombination frequencies in the *rec-1* and *rec-z* test crosses are plotted in Fig. 5. It is clear that all of the 82 *ad-7*$^+$ *asp*$^+$ progeny from cross 4805 are parental types: 65 *rec-1*$^+$ *rec-z*$^+$ and 17 *rec-1 rec-z*. There are no recombinants between *rec-1* and *rec-z*.

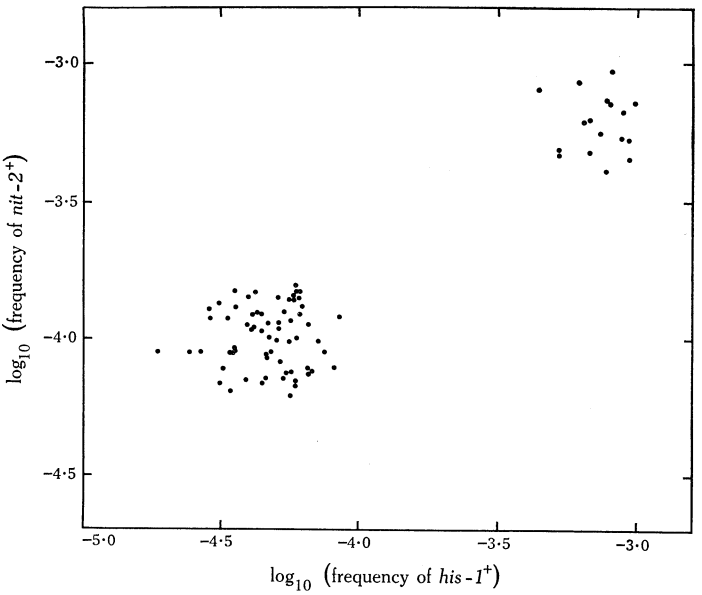

Fig. 5. Best estimates for recombination frequencies in *rec-1* and *rec-z* test crosses for the 82 *ad-7*$^+$ *asp*$^+$ recombinants from cross 4805. These were obtained by repeating crosses (see text). No recombinants between *rec-1* and *rec-z* occur in the 82 selected progeny from cross 4805; 65 are *rec-1*$^+$ *rec-z*$^+$ parental types, the remaining 17 being *rec-1 rec-z* parental types.

Map Location of rec-1

The data from cross 4805 provide a new estimate for the map location of *rec-1*; this is, in centimorgans,

Gene order and spacing	Linkage group
ad-7 (14·6) *rec-1* (3·8) *asp*	V

which is in good agreement with the original determination (D. G. Catcheside and Austin 1969).

Discussion

If *rec-1* and *rec-z* are different genes, their loci must be close together; in fact less than 0·67 map units apart with a probability of 95%. This estimate is based upon the data from cross 4805 where no progeny recombinant between the two *rec* genes were found amongst 82 selected progeny recombinant for the flanking markers *ad-7*$^+$ and *asp*$^+$ which were estimated to be 18·4 map units apart in this cross. Hence

the 82 selected progeny screened for recombination between *rec-1* and *rec-z* gave an estimate equivalent to that which would be obtained by screening 446 random progeny from cross 4805.

Although the data available at present do not constitute proof that *rec-1* and *rec-z* are the same, it must be considered highly likely; the loci are situated within the same small segment of the genome (about 0·1% of the total) and each affects recombination in an entirely analogous manner although in different chromosome segments.

Improvement of the estimate for the maximum separation of the *rec-1* and *rec-z* loci, and consequent increased confidence in the contention that the two genes are identical, could be attained by isolating and testing more *ad-7*$^+$ *asp*$^+$ recombinants from cross 4805 or by utilizing closer flankers, such as *pan-2* and *ro-4*, which are known to map between *ad-7* and *asp* (D. E. A. Catcheside 1973). However, the value of pursuing this further at present is questionable. Such a task would not only be laborious, but also faces the problem of taking into account the effect of any genetic factors operating upon recombination within the region of interest. There is good evidence for a genetic factor that modulates recombination in the *ad-7–asp* interval, since recombination values about half that in cross 4805 were observed in crosses 4369 and 5258. Since there is no basis for assigning either the high or the low frequency for *ad-7–asp* recombination as normal, it would be valid to utilize the lower estimate from cross 4369 (9·02 map units) to scale the 82 *ad-7*$^+$ *asp*$^+$ recombinants from cross 4805. In this case, the estimate for the separation between the *rec-1* and *rec-z* loci, if they are different, would be less than 0·33 map units with 95% probability. Clearly, this increase in precision is more apparent than real. The potential magnitude of the conundrum is far greater; for instance, *rec-2*$^+$ is known to reduce recombination between *pyr-3* and *his-5* from about 14% to less than 1% (Smith 1968) and combinations of genes are known to cause greater than 60-fold variation in recombination between the same pair of allelic mutations (D. E. A. Catcheside, unpublished data). Without a greater knowledge of the genetic factors influencing recombination in the vicinity of *rec-1* and *rec-z*, further use of recombination analysis to test their identity is of doubtful value.

Current evidence is consistent with *rec-1* and *rec-z* being identical; i.e. that *rec-1*$^+$ controls recombination in both the *his-1* and *nit-2* loci even though they are associated with different linkage groups. It is proposed therefore that *rec-z*$^+$ should be regarded as having defined the effect of *rec-1*$^+$ upon the *nit-2* gene. Thus the symbol *rec-z*$^+$ becomes a synonym for *rec-1*$^+$ and should no longer be used.

A pattern has emerged for the three *rec* genes, *rec-1*$^+$, *rec-2*$^+$ and *rec-3*$^+$: each is dominant in reducing recombination, each controls recombination in chromosome segments distant from its own location and it is now clear that each controls recombination in at least two segments located on different chromosomes. Nevertheless, each of the *rec* genes affects a minority of the chromosome segments on which it has been tested.

The data fit the hypothesis that the products of *rec-1*$^+$, *rec-2*$^+$ and *rec-3*$^+$ are repressors of recombination which each act to control recombination in a limited proportion of the genome, a portion that is distributed as segments, one or more genes in length, scattered on several chromosomes. One might suppose that recombination throughout the whole genome is subject to such control. If this is the case

then several other *rec* gene loci remain to be discovered. The function of the *rec* systems seems to be restricted to control of recombination; no other function has been discovered (D. E. A. Catcheside 1968). It may be advantageous for *N. crassa* to have a means of reducing recombination in limited sections of the genome. This would have the effect of stabilizing particular gene combinations without reducing the possibility of recombination throughout the genome and, more significantly, without abandoning or permanently reducing the probability of recombination, since the repressor is produced by a gene that is inherited independently. The common occurrence of variants of the *rec* genes amongst laboratory stocks and wild populations of *N. crassa* is consistent with this view.

It is clear that there must be other genetic elements in this control system, and more specifically there must be recognition sites adjacent to the chromosome segments where recombination is controlled. Two sorts of recognition site can be envisaged on theoretical grounds, one that binds the *rec* gene product (*con* for *control*) and another that binds the enzyme complex responsible for the DNA transactions of the recombination event (*cog* for *cognition*). In terms of the operon model for control of gene activity, these would be equivalent to an operator and a promoter gene respectively. However, this analogy should be treated with caution since four DNA duplexes are present, two of which must interact to instigate a recombination event.

Experimental evidence for such recognition sites is accumulating. Firstly, there is indirect evidence for *con* sites adjacent to the *his-2* and *am-1* loci. This follows from the finding that allelic recombination at these loci is differentially affected by a newly discovered *rec-3* variant (D. G. Catcheside, personal communication). Secondly, two sorts of genetic variant of sites adjacent to regulated chromosome segments have been found. These are (1) a factor close to *his-3* which is dominant in giving high frequency recombination between *his-3* alleles (Angel *et al.* 1970) and (2) a factor close to *nit-2* which is dominant in reducing recombination between *nit-2* alleles (D. E. A. Catcheside, unpublished data). These can be interpreted as variants of *cog*- and *con*-type recognition sites in the recombination control system.

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