GENES IN NEUROSPORA THAT SUPPRESS RECOMBINATION WHEN THEY ARE HETEROZYGOUS

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ABSTRACT

Genes that suppress recombination when heterozygous have been found distributed as a polymorphism in wild and laboratory populations of *Neurospora crassa.* Three alleles, ss^E, ss^S and ss^C, are associated, respectively, with the three wild types Emerson, St. Lawrence *74A* and Costa Rica *A*. It is proposed that *ss* (synaptic sequence) genes modulate recombination by determining the pairing closeness of DNA duplexes in the vicinity of the *nit-2* locus. When heterozygous, *ss* suppresses recombination 2- to 20-fold within the *nit-2* locus, which it adjoins, but crossing over in intervals flanking *nit-2* is not affected. The magnitude of suppression depends upon the *ss* alleles involved, and *ss* acts multiplicatively with *rec-1*; together, these genes modulate recombination within the *nit-2* locus over a range exceeding 100-fold. The *ss* effect is not attributable to gross chromosomal rearrangement, but could be due to small inversions or insertions, such as transposable elements.

BOTH wild and laboratory populations of *Neurospora crassa* are polymorphic for genes that influence meiotic recombination frequency in specific local regions of the genome. From these natural variants, three types of genes controlling recombination have been described; *rec* (recombination), *cog* (recognition) and *con* (control). Three *rec* loci are known: *rec-1*, *rec-2* and *rec-3*. In each case, the presence of a dominant rec^+ allele reduces recombination about an order of magnitude in several specific and scattered chromosomal locations. (ANGEL, AUSTIN and D. G. CATCHESIDE 1970; CATCHESIDE 1970, 1974; D. G. CATCHESIDE 1975; D. G. CATCHESIDE and ANGEL 1974; D. G. CATCHESIDE and CORCORAN 1973; THOMAS and D. G. CATCHESIDE 1969). One *cog* gene is known, located close to *his-3* on linkage group I, that influences recombination both within the *his-3* locus and between *his-3* and *ad-3*. The dominant allele cog^+ increases recombination up to 30-fold in the absence of *rec-2*$^+$ (ANGEL, AUSTIN and D. G. CATCHESIDE 1970). *con* loci, rendering the chromosome segments sensitive to *rec-3* control, are inferred to exist adjacent to both *am-1* on linkage group V and *his-2* on linkage group I on the basis of the differential sensitivity of allelic recombination of these two loci to the presence of the *rec-3* allele, *rec-3*^{L} (D. G. CATCHESIDE 1975).

Although genetic variants of *rec*, *con* and *cog* loci all influencing recombination within the same chromosomal segment have not yet been found, the proper-

ties of the known variants suggest that *rec-1*+, *rec-2*+ and *rec-3*+ each codes for a diffusable product that binds to *con* sites of the appropriate specificity, thereby blocking the initiation of recombination at a contiguous *cog* site by denying access of an endonuclease (ANGEL, AUSTIN and D. G. CATCHESIDE 1970; CATCHESIDE 1970, 1974; D. G. CATCHESIDE 1977).

In this paper, evidence for a fourth type of genetic factor modulating recombination in specific chromosomal segments is presented. This genetic factor has been denoted *ss* (synaptic sequence) to reflect the possibility that it is a DNA sequence influencing synapsis of specific chromosome segments. The effects ascribed to *ss* could be due to heterozygosity for transposable elements or for some other small-scale structural change. The *ss* locus is approximately 24 units distal to the mating-type locus on linkage group I. Three alleles, ss^E, ss^S and ss^C, have been found as a polymorphism among laboratory and wild strains. In crosses heterozygous for *ss* alleles, recombination in the closely linked *nit-2* locus is suppressed 2- to 20-fold, depending upon the specific *ss* alleles involved. In addition to the *ss* effect, recombination between *nit-2* alleles is reduced approximately 8-fold by the gene *rec-1*, which is located approximately 30 units from *his-1* on linkage group V (CATCHESIDE 1973, 1976). The effect of *rec-1* is selective: the dominant allele reduces allelic recombination at both the *his-1* and *nit-2* loci, but not at 24 other loci or chromosomal regions that have been tested (D. G. CATCHESIDE 1977). The effects of *ss* and *rec-1* on the *nit-2* locus are multiplicative, modulating recombination over a range exceeding 100-fold.

Genes suppressing recombination when heterozygous have also been reported in *Ascobolus immersus* (EMERSON and YU-SUN 1967; GIRARD and ROSSIGNOL 1974) and *Zea mays* (FREELING 1978). The possible roles that such genes might play in recombination is discussed, and it is proposed that *ss* determines the intimacy of synapsis of DNA duplexes in the *nit-2* region of linkage group I, close synapsis being a precondition for the initiation of a recombination event.

MATERIALS AND METHODS

Cultures: The genotype and origin of the principal cultures are detailed in Table 1. The genotype and origin of other cultures used to generate the data of Table 3 are given in a footnote to the table. In all cases, the alleles used were as follows: *al-2* (15300), *am-1* (47305), *cot-1* (C102t), *leu-3* (47313) and *un-5* (b39t). The origin of the *nit-2* mutants MN67, 68, 69 and 71 has been described (CATCHESIDE 1970). The MN103, MN105 and MN106 stocks used to obtain the data shown in Figures 4C, 4E, 4F, 5C, 5E and 5F were derived from crosses between 4407, 4409 and 4410 (the original isolates of MN103, 105 and 106, respectively, see below) and 729 or 773. The relationship between the commonly used laboratory wild-type strains of Neurospora, including Emerson and St. Lawrence, is discussed by BARRATT (1962) and D. G. CATCHESIDE (1975).

Media, crossing methods and recombination assays: These were as originally described by CATCHESIDE (1970, 1974, 1979). Crosses were made by mixing dense conidial suspensions in 150 × 15 mm tubes containing 4 ml of Westergaard medium (WESTERGAARD and MITCHELL 1947), modified by the addition of 6.25 mM NH_4NO_3 in place of KNO_3 and supplemented with 2% sucrose, 5 mM L-alanine, and a 6 cm spill folded from a 6 × 4 cm sheet of Whatman No. 1 filter paper. Crosses were incubated at 25° for ⩾ 30 days.

The frequency of recombination between *nit-2* alleles was determined by measuring the frequency of formation of *nit-2*+ recombinants. This is possible in crosses homozygous for *am-1*, as

TABLE 1

Genotype and origin of principal cultures

Stock number	Source†	Known genotype
5	DGC 11	251/12 *a* wild type
6	DGC 4	Beadle 312.55 *a* wild type
9	DGC (= FGSC 740)	Chilton *a* wild type
96	J. R. S. FINCHAM	St. Lawrence *74A* wild type
729	DGC 5901	*a rec-3; cot-1; rec-1*
773	DGC 3492	*a rec-3 al-2; am-1 rec-1*+
817	DGC 3673	*A rec-3*+ *al-2; cot-1; am-1 rec-1*+
855	*	*ss*^{E} *A; cot-1; am-1 rec-1*
1199	*	*ss*^{E} *nit-2(MN73) A rec-3 al-2; cot-1; am-1 rec-1*
1373	*	*ss*^{E} *nit-2(MN73) A rec-3 al-2; cot-1; am-1 rec-1*+
1693	*	*ss*^{E} *nit-2(MN72) a rec-3*+ *al-2; cot-1; am-1 rec-1*+
1871	*	*ss*^{E} *nit-2(MN70) A rec-3 al-2; cot-1; am-1 rec-1*
2159	*	*ss*^{E} *nit-2(MN70) a rec-3 al-2; cot-1; am-1 rec-1*
2202	*	*ss*^{E} *nit-2(MN73) a rec-3 al-2; cot-1; am-1 rec-1*+
2207	*	*ss*^{E} *nit-2(MN73) a rec-3 al-2; cot-1; am-1 rec-1*
2244	a	*ss*^{S} *nit-2(nr37) a; cot-1; am-1 rec-1*+
2876	b	*ss*^{S} *nit-2(nr37) a; cot-1; am-1 rec-1*+
2951	c	*ss*^{S} *nit-2(nr37) a al-2; cot-1; am-1 rec-1*
3393	FGSC 851	Costa Rica *A* wild type
3399	FGSC 961	Liberia *UA1 A* wild type
3737	d	*ss*^{S} *A; cot-1; am-1 rec-1*
3955	FGSC 539	*leu-3 a*
3988	e	*ss*^{E} *nit-2(MN70) A rec-3 al-2; cot-1; am-1 rec-1*+
4087	f	*a al-2; cot-1; am-1 rec-1*+
4088	g	*A al-2; cot-1; am-1 rec-1*+
4089	h	*a; cot-1; am-1 rec-1*+
4093	i	*ss*^{C} *A; cot-1; am-1 rec-1*
4094	j	*A; cot-1; am-1 rec-1*
4201	FGSC 1316	*un-5 A*
4333	k	*ss*^{E} *nit-2(MN70) leu-3 a; cot-1; am-1 rec-1*
5557	l	*un-5 ss*^{S} *nit-2 (nr37) A al-2; cot-1; am-1 rec-1*
5646	m	*ss*^{E} *nit-2(MN72) leu-3 a; cot-1; am-1 rec-1*
6297	n	*ss*^{E} *nit-2(MN70) a; cot-1; am-1 rec-1*+
9891	o	*ss*^{E} *a; cot-1; am-1 rec-1*
9892	o	*ss*^{E} *a; cot-1; am-1 rec-1*
9893	o	*ss*^{E} *a; cot-1; am-1 rec-1*
9899	p	*ss*^{C} *a; cot-1; am-1 rec-1*
9900	p	*ss*^{C} *a; cot-1; am-1 rec-1*
9901	q	*ss*^{S} *a; cot-1; am-1 rec-1*
9902	r	*ss*^{S} *a; cot-1; am-1 rec-1*

† Sources of stocks were as follows: DGC, D. G. CATCHESIDE (the origin of DGC 4 and DGC 11 is not clear, but they are probably derived from intercrosses of Lindegren *25a* and *1A* by Beadle); FGSC, the Fungal Genetics Stock Center; *, described in CATCHESIDE 1970; a, from cross 2112*; b, from 855 × 2244; c, from cross 2655*; d, from 96 × 2207; e, from 773 × 1196*; f, from 5 × 1373; g, from 6 × 1373; h, from 9 × 1373; i, from 2207 × 3393; j. from 2207 × 3399; k, from 1199 × 3955; l, from 2951 × 4201; m, from 1885* × 3955; n, from 817 × 2160 (an *MN70 a* isolate from 773 × 1196*); o, from 855 × 1374*; p. *nit-2(MN103)* was introduced into 4093 by mutation and a stock 8918: *nit-2(MN103) a rec-3 al-2; cot-1; am-1 rec-1* was made by crossing the mutant to 773 and extracting a *nit-2* isolate, which was then crossed to 729; 9899 and 9900 are progeny from 4093 × 8918; q, from 3737 × 2949 (2949 is a sibling of 2951 having the same genotype); r, from 3737 × 2951.

nit-2 mutants are unable to derepress NAD-linked glutamate dehydrogenase, and *am-1* is the structural gene for NADP-linked glutamate dehydrogenase. Consequently, only *nit-2*+ *am-1* recombinant progeny can grow on minimal medium, while both recombinant and parental types grow in the presence of an α-amino nitrogen source, such as alanine. Crosses were also made homozygous for the colonial temperature-sensitive mutant *cot-1*, in order to permit high plating densities. The bulk of the spores from a crossing tube were suspended in VOGEL's "N" minimal medium (VOGEL 1964) containing 2% sucrose and 0.6% Bacto-Agar (Difco) and heat-shocked at 60° for 60 min. Samples of 3 ml were plated on each of five 20 ml plates of VOGEL's medium containing 0.5% sorbose, 0.1% sucrose and 2% Bacto-Agar. In crosses yielding very high frequencies of *nit-2*+ recombinants, a 1-in-20 dilution was also plated on sorbose-sucrose medium. The number of viable spores was estimated by plating three 3 ml samples of a 1-in-800 dilution on 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° and then transferred to 34° for 48 hr prior to counting colonies. Alanine-supplemented plates were incubated for 18 hr at 25°, followed by 24 hr at 34°. The temperature jump restricts growth and elicits thickening of the colonies, due to the presence of *cot-1*. This aids visibility of the colonies and enables counting up to 500 colonies per plate. Reversion of *am-1* (47305) occurs with a frequency $< 10^{-8}$, and does not interfere with the assay.

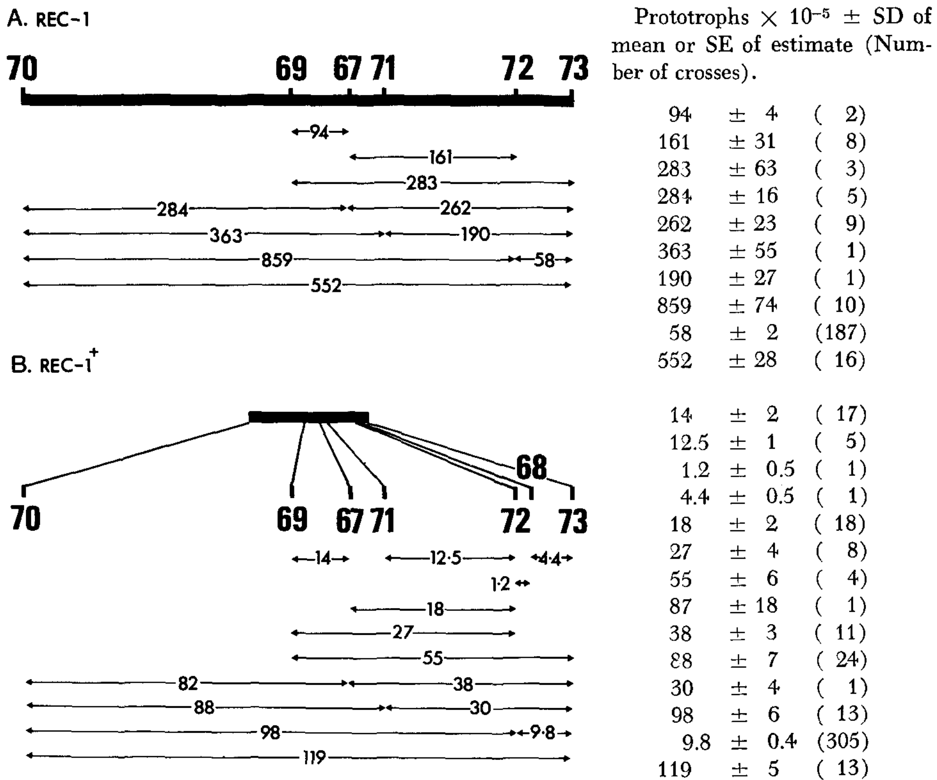
The frequency of white, aborted ascospores was determined by counts in several randomly selected microscope fields of shot spores adhering to the wall of the crossing tube.

Isolation of new nit-2 *mutants*: New *nit-2* mutants were isolated by filtration enrichment on minimal medium following UV mutagenesis of strains containing *am-1* (CATCHESIDE 1970). *nit-2* mutants MN95, 96, 97 and 98 were induced in 3737, which contains the *nit-2*+ gene of St. Lawrence *74A*. Mutants MN103, 105, 106 and 107 were induced in 4093, which has *nit-2*+ from Costa Rica *A*. Mutants MN111, 112, 115 and 116 were isolated respectively in 4087, 4089, 4094 and 4088 containing *nit-2*+ from 251/12 *a*, Chilton *a*, Liberia *UA1 A* and Beadle *a*.

RESULTS

St. Lawrence and Emerson wildtypes differ in a genetic factor modulating recombination between nit-2 *alleles*: *nit-2* mutants MN67, MN68, MN69, MN70, MN71, MN72 and MN73 were induced in strain 855 (CATCHESIDE 1970), which has a *nit-2*+ locus derived from the Emerson wild types, most probably from Emerson *A* 1534; $P \simeq 0.8$ (D. G. CATCHESIDE, unpublished records). Crosses between pairwise combinations of these alleles, both in matings homozygous *rec-1* and heterozygous *rec-1 rec-1*+, yield frequencies of prototrophic recombinants that can be represented as a one-dimensional map of the locus (Figure 1). The interstitial distances on the maps are approximately additive.

The MN67–MN73 mutants were selected originally on the basis of their ability to suppress leaky growth of *am-1* mutants and were only subsequently shown to be alleles of the *nit-2* locus. Previously, *nit-2* mutants had been shown to be deficient in nitrate reductase (SORGER and GILES 1965), and their pleiotropic effects on nitrogen metabolism were not known. Experiments to test the hypothesis that the MN67–73 mutants are *nit-2* alleles included making crosses between them and an authentic *nit-2* allele, nr37 (*nit-2* nr37 was isolated by SORGER and GILES in the St. Lawrence 74A wild type). The recombination data (Figure 2) supported the hypothesis that MN67–73 are *nit-2* alleles; nr37 is in close proximity to MN73. However, the frequency of prototrophic recombinants in crosses between nr37 and any of MN67–73 was found to be



Prototrophs $\times 10^{-5} \pm$ SD of mean or SE of estimate (Number of crosses).

A		
94	± 4	(2)
161	± 31	(8)
283	± 63	(3)
284	± 16	(5)
262	± 23	(9)
363	± 55	(1)
190	± 27	(1)
859	± 74	(10)
58	± 2	(187)
552	± 28	(16)

B		
14	± 2	(17)
12.5	± 1	(5)
1.2	± 0.5	(1)
4.4	± 0.5	(1)
18	± 2	(18)
27	± 4	(8)
55	± 6	(4)
87	± 18	(1)
38	± 3	(11)
88	± 7	(24)
30	± 4	(1)
98	± 6	(13)
9.8	± 0.4	(305)
119	± 5	(13)

FIGURE 1

FIGURE 1.—Linkage map of the *nit-2* locus based on data from crosses between alleles isolated in the Emerson wild type. (A) crosses homozygous for *rec-1*. (B) crosses containing *rec-1*+. Allele designations are abbreviated by omitting the MN prefix.

The data represent a set collected only in part for the specific purpose of constructing a map of the *nit-2* locus. Much of the data is gleaned from various experiments concerned with determining the properties and location of the *rec-1* gene. Hence, the recombination frequencies for a particular allele pair are derived only in part from repeated estimates of the frequency obtained by crossing two specific stocks, and are derived mainly from crosses between several or many different pairs of stocks of the appropriate genotype. Since these stocks are related by a complex pedigree, this has not been presented here. There is some merit in constructing the maps from such a set of isolates, since the heterothallic nature of *N. crassa* virtually precludes the construction of heteroallelic stock with isogenic backgrounds, and the use of a minimum number of stocks of the genotypes required to obtain all heteroallelic pairs may result in biased estimates of recombination frequency due to the differential presence of genes having a small effect on recombination. A gene of this nature changing recombination between *nit-2* alleles by about 1.5-fold is known (CATCHESIDE 1970, 1974) but impractical to assay. The approach to mapping used here minimizes the confounding influence of such genes. It is probable that the aberrantly high yield of recombinants in crosses between MN70 and MN72 is due to segregation of this gene.

aberrantly low (Figure 2). For example, in the presence of *rec-1*, crosses between MN70 and MN73 yield *nit-2*+ recombinants with a frequency of (552 ± 28) × 10^{-5} (Figure 1); yet, crosses of MN70 × nr37 yield (107 ± 5) × 10^{-5} *nit-2*+, and crosses of nr37 × MN73 yield (1.7 ± 0.3) × 10^{-5}*nit-2*+ (Figure 2), a combined frequency of only ~(109 ± 5) × 10^{-5}. This is about 20% of the recombination frequency expected. A similar reduction is found in the segment of the *nit-2* gene bounded by MN67 and MN73, in both the presence and absence of *rec-1*+ (Figure 2).

In order to determine whether the shortfall of *nit-2*+ recombinants in crosses containing the nr37 allele was an allele-specific effect or reflected a genetic difference between the Emerson and St. Lawrence wild types, a set of new *nit-2* mutants, MN95–98, was induced in strain 3737. This strain has the *nit-2*+ region of linkage group I derived from St. Lawrence *74A*. Two of the alleles from this series have been crossed to MN70 and MN73. These St. Lawrence alleles are mutant at sites within the *nit-2* locus widely separated from nr37, yet both behave like nr37, giving aberrantly low recombination frequencies with the Emerson alleles (Table 2). The effect observed with nr37 is thus not allele specific, but is due to an inherent genetic difference between the St. Lawrence *74A* and Emerson wild types that influences recombination within the *nit-2* locus. More-

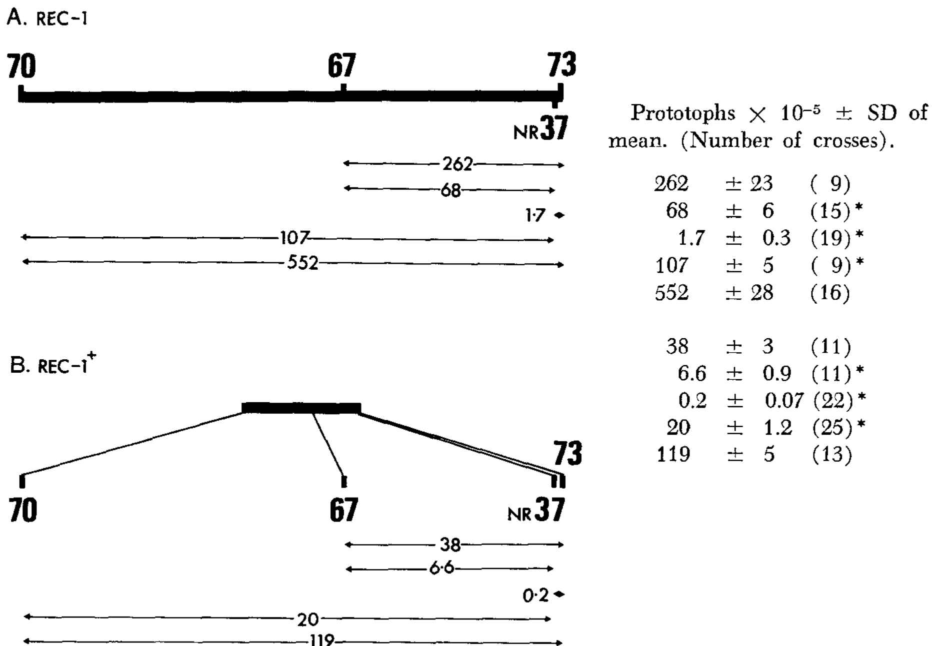


FIGURE 2

FIGURE 2.—Partial linkage map of the *nit-2* locus showing aberrant recombination frequencies in crosses involving allele nr37. (A) crosses homozygous for *rec-1*. (B) crosses containing *rec-1*+. Recombination values from crosses containing nr37 are marked with an asterisk. Allele numbers are abbreviated by omitting the MN prefix.

TABLE 2

Aberrantly low recombination frequencies in crosses between nit-2 *alleles derived from Emerson and St. Lawrence wild types*

St. Lawrence allele	*rec-1* constitution of cross	Prototroph frequency $\times 10^{-5}$ in crosses to Emerson alleles*		Recombination in the MN70 MN73 interval as a percentage of that expected†
		MN70	MN73	
MN96	*rec* × *rec*	75 ± 5 (19)	24 ± 2 (13)	17.9
	rec × *rec*+	18.2 ± 1.6 (24)	4.3 ± 0.5(15)	19.0
MN98	*rec* × *rec*	4.8 ± 1.5 (5)	88 ± 5 (24)	16.8
	rec × *rec*+	0.53 ± 0.09(4)	15.4 ± 0.8(23)	13.4

* Prototroph frequency ± standard deviation of the mean in (n) crosses. The data represent the mean of values obtained (Table 3) in crosses between several different stocks of the requisite genotype, not the mean of replicates of the same cross.

† The sum of recombination between the St. Lawrence allele and each of MN70 and MN73 as a percentage of the recombination observed in crosses between MN70 and MN73 (see Figure 2 and Table 3).

over, the effect is not due to *rec-1*; recombination frequencies are aberrantly low both in crosses homozygous for *rec-1* and in crosses heterozygous for *rec-1*/*rec-1*+, being 18.5 ± 1.3% (n=8) of that expected when calculated from the data in Figure 2 and Table 2. The effect is independent of the *rec-1* constitution of crosses, being multiplicative with the reduction in recombination between *nit-2* alleles occasioned by the presence of *rec-1*+. The genes determining this effect have been designated *ss*, the allele present in St. Lawrence being ss^S and the allele in Emerson being ss^E.

The map location and dominance relationship of ss *alleles*: The first indication of the map location of *ss* genes came from a cross designed to test the hypothesis that *nit-2* nr37 is like the Emerson alleles in being subject to the influence of *rec-1*+ in reducing recombination. The data are published (Figure 4 in CATCHESIDE 1970; however, the analysis did not consider the *ss* genes. Analysis that takes account of the *ss* genes is now possible. Stock 855, which has the genotype ss^E *A cot-1 am-1 rec-1*, was crossed to stock 2244, which has the genotype ss^S *nit-2* (nr37) *a cot-1 am-1 rec-1*+, and *nit-2* progeny were selected and crossed to *rec-1* testers of genotype ss^E *nit-2*(MN70) *a*/*A cot-1 am-1 rec-1*. The frequency of *nit-2*+ recombinants was determined in these crosses and only two groups were found: 57 progeny giving a mean of 0.21×10^{-3} recombinants with a standard deviation of 0.45×10^{-4}, and 54 progeny giving a mean of 1.73×10^{-3} with a standard deviation of 4.4×10^{-4}. The two frequency groups reflect the segregation of *rec-1*+, which is not linked to *nit-2*. If ss^E *nit-2*(nr37) recombinants had been obtained, then two further groups of prototroph frequency would be expected: a group with a mean $\sim 1.1 \times 10^{-3}$ and another with a mean of $\sim 9.4 \times 10^{-3}$, since crosses heterozygous for ss^E/ss^S yield approximately 18.5% of the recombinants observed in crosses homozygous for ss^E (Table 2, Figure 2). On the basis of the available data, any ss^E *nit-2*(nr37) *rec-1*+ recombinants cannot be distinguished from the parental class: ss^S *nit-2*(nr37) *rec-1*. Hence, only half of the recombinants, the ss^E *nit-2*(nr37) *rec-1* class, could be detected. Since no

such recombinants were found, the data imply that the *ss* locus is on linkage group I and, with 95% probability, within 5.3 map units of the *nit-2* locus.

In order to determine the dominance relationships of the *ss* alleles, it was desirable to obtain a recombinant between the *ss* and *nit-2* loci so that the effect of homozygosity and heterozygosity of *ss* alleles on recombination within the *nit-2* locus could be assessed, using the same pairs of *nit-2* alleles in each case. Recombinants between *nit-2* and *ss* have not been obtained. They were sought in the following way: to improve the efficiency, only recombinants between the closest available markers flanking the *nit-2* region were examined. The following crosses, each of which was homozygous for *rec-1*, *am-1* and *cot-1*, were set up:

Number	Parents	Genotype
5720	5646(ss^E *MN72**) × 5557(ss^S *nr37*)	+ *nit-2** *leu-3* *a*
6586	4333(ss^E *MN70**) × 5557(ss^S *nr37*)	*un-5* *nit-2* *nr37* + *A*

Samples of ascospores were plated on alanine-supplemented medium at 34° to select un^+ leu^+ recombinants and on alanine- and leucine-supplemented medium at 25° to estimate the number of viable ascospores. The total recombination between *un* and *leu-3* was found to be 19.4 ± 1.4% (8 samples) for cross 5720 and 24.6 ± 1.1% (3 samples) for cross 6586. For cross 5720, 155 un^+ leu^+ *A* recombinant progeny were selected at random and crossed to stock 2876 (*nit-2*(nr37) *a*; *rec-1*$^+$) and stock 1693 (*nit-2*(MN72) *a*; *rec-1*$^+$). Analysis of the frequency of *nit-2*$^+$ recombinants in these crosses (0 or 4.1 ± 0.6 × 10^{-5}) enabled the determination of which *nit-2* allele they carried; 114 were *nit-2*(MN72) and 41 *nit-2*(nr37). For the nr37 isolates, these crosses allow determination of the *ss* genotype. However, the close linkage of nr37 and MN72, and only moderate spore yields, made this determination equivocal. Therefore, the *ss* genotype of all of the un^+ leu^+ progeny was determined by measuring the frequency of *nit-2*$^+$ recombinants in crosses to the tester 6297 (*nit-2*(MN70) *a*; *rec-1*$^+$). For the *nit-2* (MN72) progeny, these crosses are expected to be homozygous ss^E and yield a high frequency of recombination relative to any MN72 ss^S recombinants. Only one class of recombination frequency, that expected for ss^E homozygotes, was observed (Figure 3A). For the *nit-2*(nr37) isolates, the testcrosses are expected to be heterozygous ss^E/ss^S and yield a low frequency of recombination relative to any nr37 ss^E recombinants. Here again only one class of recombination frequency, that expected for ss^E/ss^S heterozygotes, was observed (Figure 3B).

For cross 6586, 226 un^+ leu^+ *A* recombinant progeny were selected at random and crossed to stocks 2876 and 6297. Analysis of the frequency of *nit-2*$^+$ recombinants in these crosses permitted identification of the *nit-2* allele carried: 136 were *nit-2*(MN70) and 90 *nit-2*(nr37). For the nr37 isolates, these crosses also allowed determination of their *ss* genotype (Figure 3D). No recombination events between the *ss* and *nit-2* loci were detected. The *ss* genotype for 108 of the

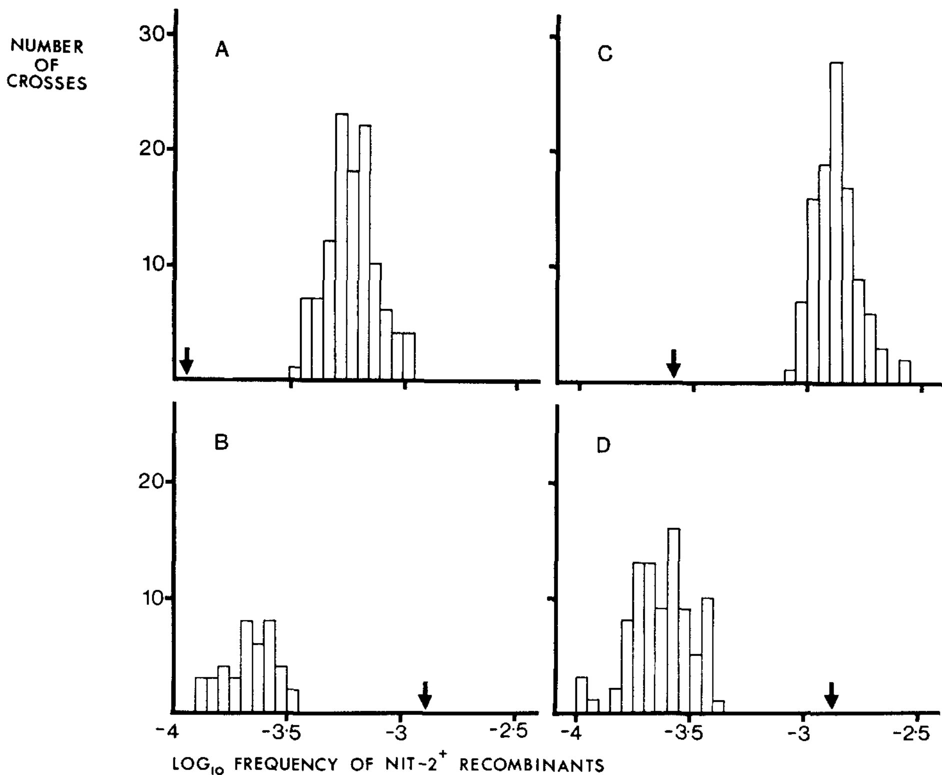


FIGURE 3.—Frequency of *nit-2+* recombinants in testcrosses designed to detect progeny from crosses 5720 and 6586 that are recombinant for *ss* and *nit-2*. The arrows indicate the *nit-2+* frequency expected of *ss nit-2* recombinants, calculated on the basis that *nit-2+* frequency in crosses heterozygous for ss^E/ss^S is 0.185 of that in crosses homozygous for ss^E (Table 2; Figure 2). No such recombinants were found. (A) MN72 progeny from cross 5720 × 6297 (ss^E MN70 *rec-1+*). (B) nr37 progeny from cross 5720 × 6297. (C) MN70 progeny from cross 6586 × 2202 (ss^E MN73 *rec-1+*). (D) nr37 progeny from cross 6586 × 6297.

MN70 isolates was determined by crossing them to stock 2202 (*nit-2*(MN73) *a*; *rec-1+*). Again, no recombinants between the *nit-2* and *ss* loci were obtained (Figure 3C) and the attempt to obtain such recombinants was terminated.

Combining the data from crosses 5720 and 6586, the equivalent of 1719 random progeny have been screened for recombination between the *ss* and *nit-2* loci in the region distal to *nit-2* and the equivalent of 1530 random progeny for the region proximal to *nit-2*. Since no such recombinants were found, the *ss* locus is within 0.20 map units proximal and 0.17 map units distal to *nit-2*, with 95% probability. This interval should be compared with the size of the *nit-2* locus, which is 0.10 map units in the same heterozygous ss^E/ss^S genetic background (Figure 5D).

As recombinants between *ss* and *nit-2* could not be obtained, a less direct approach to determining the dominance relationships of ss^E and ss^S was used.

Three *nit-2* alleles derived from Emerson were chosen, as were another three derived from St. Lawrence wild type. The alleles were selected for their distribution in the *nit-2* locus, one as near each end as possible and one other. Stocks enabling crosses to be made between all possible allele pairs were constructed. The stocks permitted such crosses to be made both homozygous for *rec-1* and also containing *rec-1*$^{+}$ (Table 3). Recombination maps of the *nit-2* locus were constructed from these data (Figure 4A, B and D and Figure 5A, B and D). The maps constructed from recombination data obtained from crosses heterozygous for genetic background and hence heterozygous ss^{E}/ss^{S} (Figures 4D and 5D) are substantially shorter than maps constructed from crosses homozygous for either ss^{E} or ss^{S} (Figures 4A, B and 5A, B). Heterozygosity for the alleles ss^{E} ss^{S} suppresses recombination in the *nit-2* locus. It should also be noted that crosses homozygous for genetic background do not yield maps of similar length of the *nit-2* locus (Figures 4A, B and 5A, B). Differences in the length of the *nit-2* maps based upon crosses homozygous for different *ss* alleles are consistent with the hypothesis proposed in the DISCUSSION.

The distribution of ss genes among Neurospora wild types: *nit-2* mutants have also been induced in a range of other wild types, and these have been crossed to the Emerson alleles MN70 and MN73 in order to check for the presence of genetic factors modulating recombination (Table 4). The data for Costa Rica, Chilton and Liberia *A* are consistent with their possessing an *ss* allele other than ss^{E}. The results for 251/12 and Beadle were equivocal in view of the scanty data, and these wild types may well contain the ss^{E} allele. The genetic factor in Costa Rica has been investigated further. Three *nit-2* alleles isolated in Costa Rica were selected: numbers MN103, MN105 and MN106. Stocks were constructed such that crosses could be set up among these alleles in all pairwise combinations, among these three alleles and each of the Emerson alleles MN67, MN70 and MN73 in all pairwise combinations, and also between the three Costa Rica alleles and the St. Lawrence alleles nr37, MN96 and MN98, again in all pairwise combinations. The stocks allowed all of these crosses to be performed both homozygous for *rec-1* and also containing *rec-1*$^{+}$. As in the case of Emerson and St. Lawrence, the *nit-2* locus maps constructed from recombination data obtained from crosses heterozygous for the genetic backgrounds of Emerson and Costa Rica (Figures 4E and 5E) are substantially shorter than the maps constructed from crosses homozygous for either Emerson or Costa Rica (Figure 4A, C and Figure 5A, C). Moreover, the conjoint maps of the *nit-2* locus constructed from crosses between Costa Rica and St. Lawrence alleles also are short in comparison with the maps derived from crosses homozygous for either genetic background (Figure 4F, Figure 5F). The *ss* allele present in Costa Rica thus differs from both ss^{E} and ss^{S}; it has been designated ss^{C}. Each of the three pairwise heterozygous combinations of these alleles suppresses recombination within the *nit-2* locus below the frequencies observed in any of the three classes of homozygotes. The effect is essentially independent of the action of *rec-1*$^{+}$ in reducing recombination between *nit-2* alleles (*cf.* Figure 4 and Figure 5).

TABLE 3

Recombination frequency observed in crosses between all pairwise combinations of two sets of nit-2 *alleles, one (MN67, 70, 73) derived in Emerson wild type* (ss^E) *and the other (nr37, MN96, 98) in St. Lawrence wild type* (ss^S)

Allele pair	*rec* constitution	Stock numbers and crosses	Prototroph frequency × 10^{-5}
MN67 × 70	*rec*	1868 × 1871, 1872, 3990	322, 288, 282 226 303
	rec/+	2151 × 3990	127 65 55
MN67 × 73	*rec*	1868 × 1199, 2311, 1372; 2153 × 1199; 1867 × 2207	228 222 222, 342, 250; 202 203 401; 285
	rec/+	1868 × 1373; 2151 × 1199, 2311, 1372; 1867 × 2202;	50; 46 37 31 23, 34, 53; 42;
		2155 × 1199	40
	+	2151 × 1373	33 33
MN70 × 73	*rec*	1374 × 3990; 2207 × 1196, 1871, 1872, 3990	734 458 354; 738, 493 584 710 586 510 512, 485, 654 573 570 397 476
	rec/+	2202 × 1196, 1871, 1872, 3990;	125, 171 120 118 117 113, 99, 102 112 130;
		2207 × 3988, 3989, 3992	114, 122, 100
MN98 × nr37	*rec*	3886 × 2949, 2951, 2990, 2991, 2996;	256 230 242 209 229, 230 186 127, 168 176, 178 267, 277;
		8352 × 2949, 2952, 2990;	315, 174, 101 426;
		8355 × 2952, 2953, 2994, 2876; 8353 × 2949, 2990	171, 277 288, 367 191, 178 176; 224 245, 173 226
	rec/+	3886 × 2876, 2877, 2950, 2954;	38 46, 36 42, 38, 35 34 19;
		8352 × 2244, 2876, 2877;	45 40, 44 65, 34 44;
		8355 × 2245, 2246, 2247, 2248, 2249, 2251, 2956;	31 79, 35 40, 46 54, 33 101, 35 50, 81 32, 36 41;
		8354 × 2951, 2990, 2991, 2996;	46 47, 43 48, 50 45, 43 47;
		8353 × 2244, 2876, 2877	24 33, 30 35, 31 36
	+	8354 × 2244, 2876, 2877, 2949, 2950, 2954, 2955, 2988, 2989, 2992, 2993	57 40, 49 51, 27 51, 43 41, 56 49, 44 52, 54 35, 34 42, 42 69, 52 54, 37 35
MN96 × nr37	*rec*	8105 × 2879, 2952, 2953, 2994, 2995; 8106 × 2879	25 45, 36 25, 48, 33 52, 36; 18
	rec/+	8101 × 2877; 8102 × 1868, 2949; 8103 × 2990;	7; 6, 13; 7;
		8104 × 2879, 2952	16 5, 5 9
	+	8100 × 2876, 2992; 8104 × 2245, 2246, 2247, 2248, 2249, 2251, 2954, 2956	2, 2; 10 10, 6 13, 14 20, 11, 16 20, 8 10, 7, 17

TABLE 3—Continued

Allele pair	*rec* constitution	Stock numbers and crosses	Prototroph frequency × 10^{-5}
MN96 × 98	*rec*	8355 × 8101, 8103; 8105 × 3886, 8352, 8353;	166, 124 116; 80 148, 82 114, 75, 96;
		8106 × 3886, 8352, 8353	95 147, 68 87, 93 63
	rec/+	8104 × 3886, 8352, 8353; 8354 × 8105, 8106;	28 50, 53, 39 37; 35 43, 25;
		8355 × 8100, 8102	28 33, 6 14
MN70 × 98	*rec*	3886 × 2159; 3990 × 7449.7	8 1 4 9; 2
	rec/+	3886 × 6297; 3989 × 7449.7	0.8 0.5 0.4; 0.4
MN70 × 96	*rec*	2159 × 3880, 4861.2, 4861.5; 3990 × 4861.12,	70 100, 85, 98; 110,
		4861.13, 4861.15; 8101 × 2159, 2161;	76, 94; 60 72, 66 74;
		8103 × 2159, 2161; 8105 × 1196, 1871	58 59, 62 130; 58 49, 58 48
	rec/+	6297 × 3880, 8101, 8103, 4861.2, 4861.5;	18 18, 19, 18 20, 44, 32;
		6298 × 8101, 8103; 8105 × 3988, 3992;	16 11, 17 18; 11 18, 12 11;
		3989 × 4861.12, 4861.13, 4861.15;	15, 11, 11;
		2159 × 4861.1, 4861.4; 3990 × 4861.3	13, 21; 18
	+	6297 × 4861.1, 4861.4; 3989 × 4861.3	29, 18; 19
MN70 × nr37	*rec*	2159 × 2879, 2952, 2953	105 102 108, 104 107 145, 90 111 94
	rec/+	6297 × 2879, 2952, 2953; 2159 × 2245, 2246,	27 26 20, 26 20 20, 33 28 19; 14, 30 22 24,
		2247, 2248, 2249, 2251; 2161 × 2245, 2246,	22, 18, 19, 19; 16, 20,
		2247, 2248, 2249, 2251	16, 12, 20, 10 13 12
MN67 × 98	*rec*	3886 × 1868, 2153	62 72 29, 58 36 29
	rec/+	3886 × 2151	5 11 12
MN67 × 96	*rec*	8103 × 2153; 8105 × 1193, 1867; 8106 × 1193	15; 16, 19; 13 13
	rec/+	8104 × 1193, 1867	1, 3 2
MN67 × nr37	*rec*	1868 × 2952, 2953, 2879; 2153 × 2953, 2879	47 68 91, 114 73 47, 100 63 47; 72 57 45, 96 61 34
	rec/+	1868 × 2245, 2247, 2248, 2249, 2251; 2151 × 2952,	9, 2, 5, 4, 9; 10 10 3,
		2953	8 5 7

TABLE 3—Continued

Allele pair	*rec* constitution	Stock numbers and crosses	Prototroph frequency × 10^{-5}
MN73 × 98	*rec*	1199 × 7449.7, 8355; 1374 × 3886, 8352, 8353; 2207 × 3886, 7449.1, 7449.2, 7449.4, 7449.5, 7449.10, 7449.12, 8352, 8353	108, 71 79; 90 55 36, 87 70 95, 66 68; 62 103 153 133, 98, 113, 129, 111, 81, 89, 80, 70 67
	rec/+	1373 × 7449.7, 8355; 1374 × 8354; 2202 × 3886, 8352, 7449.1, 7449.2, 7449.4, 7449.5, 7449.10, 7449.12; 2205 × 8352; 2207 × 7449.3, 8354	18, 16 18; 13 21; 11 15 18 3, 13 16 21, 16, 19, 17, 14, 11, 16; 19; 16, 13 16
	+	2202 × 7449.3	15
MN73 × 96	*rec*	1199 × 4861.13, 4861.15, 8105; 1374 × 8101, 8103; 2207 × 4861.2, 4861.5, 8101, 8103	28, 35, 25; 26 24, 15 25; 35, 32, 20 19, 11 20
	rec/+	1199 × 4861.3; 1373 × 4861.13, 4861.15, 8105; 2202 × 4861.2, 4861.5, 8101, 8103; 2205 × 8101, 8103; 2207 × 4861.1	5; 1, 4, 7; 8, 1, 3 6, 4; 3 5, 5; 5
	+	1373 × 4861.3; 2202 × 4861.1	5; 3
MN73 × nr37	*rec*	1374 × 2879, 2953; 2207 × 2879, 2952, 2953	2 0.7 1.1, 1.4 1.2 1.6; 5.2 1.9 3.4 1.5 2.1 0.6 0, 0 1.9 1.9, 1.1 2.8 2.6 0
	rec/+	2202 × 2879, 2952, 2953; 2207 × 2245, 2246, 2247, 2248, 2249, 2251	0 0 0 0 0 0 0.5, 1, 0.9 0.3; 0.3, 0, 0, 0.6, 0, 0
	+	2202 × 2245, 2246, 2247, 2248, 2249, 2251, 2955	0, 0.6, 0.4, 0, 0, 0, 0

Genotype and origin of the stocks used that are not included in Table 1 (all contain *am-1* 47305 *cot-1* C102t; the origin of stocks marked with an asterisk is given in CATCHESIDE 1970):

ss^E *MN67* : *A rec-1*: 1193* 1867, *a rec-1*: 1868 2153, *a rec-1*+: 2151 2155 (1193 is the original isolate of MN67. Other stocks are siblings from 1193 × 773).

ss^E *MN70* : *A rec-1*: 1196* 1872 3990, *A rec-1*+: 3989 3992, *a rec-1*: 2161, *a rec-1*+: 6298 (1196 is the original isolate of MN70. 6298 is from a cross between a *nit-2* (MN70) *a am-1* (47305) isolate from 1196 × 773 crossed with 817. Other stocks are siblings from 1196 × 773).

ss^E *MN73* : *A rec-1*: 1372, *a rec-1*: 1374, *a rec-1*+: 2205 (all from a cross 1199* × 773).

ss^S *nr37* : *A rec-1*: 2879 2952 2953 2994 2995, *A rec-1*+: 2245 2246 2247 2248 2249 2251 2954 2956, *a rec-1*: 2877 2949 2990 2991 2996, *a rec-1*+: 2950 2955 2988 2989 2992 2993 (2245 to 2251 are from a cross 1218* × 2057*, 2877 to 2996 are siblings from 855* × 2244).

ss^S *MN96* : A *rec-1*: 3880 4861.2 4861.5 8101 8103, *A rec-1*+: 4861.1 4861.4 8100 8102, *a rec-1*: 4861.12 4861.13 4861.15 8105 8106, *a rec-1*+: 4861.3 8104 (3880 is the original isolate of MN96. Other stocks are siblings from 3880 × 773).

ss^S *MN98* : *A rec-1*: 3886 7449.1 7449.2 7449.4 7449.5 7449.10 7449.12 8352 8353, *A rec-1*+: 7449.3 8354, *a rec-1*: 7449.7 8355. (3886 is the original isolate of MN98. Other stocks are siblings from 3886 × 1218*).

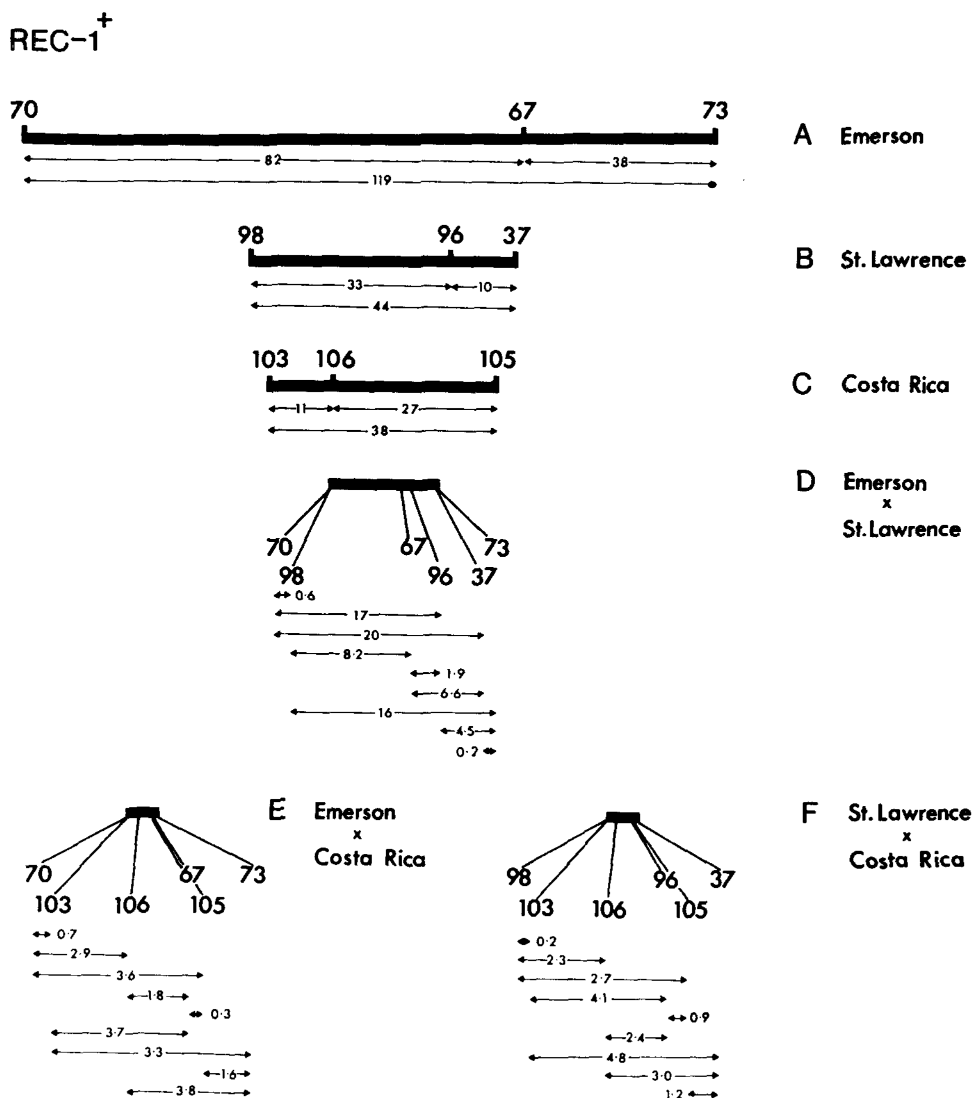


FIGURE 4.—Maps of the *nit-2* locus based upon crosses between mutants isolated in various wild types and containing *rec-1*+. The crosses are between: (A) Emerson alleles, homozygous ss^E; (B) St. Lawrence alleles, homozygous ss^S; (C) Costa Rica alleles, homozygous ss^C; (D) Emerson and St. Lawrence alleles heterozygous ss^E/ss^S; (E) Emerson and Costa Rica alleles, heterozygous ss^E/ss^C; (F) St. Lawrence and Costa Rica alleles, heterozygous ss^S/ss^C.

The fiducial limits for the recombination frequencies in 4A, 4B and 4D are calculable from the data in Table 3. For the remainder they are as follows (mean ± SD of mean or SE of estimate (number of crosses analyzed)): 4C: 11 ± 0.7(3), 27 ± 4(3), 38 ± 5(4). 4E: 0.7 ± 0.2(3), 2.9 ± 0.5(9), 3.6 ± 0.4(3), 1.8 ± 0.6(9), 0.3 ± 0.2(6), 3.7 ± 0.9(3), 3.3 ± 0.4(12), 1.6 ± 0.2(19), 3.8 ± 0.4(22). 4F: 0.2 ± 0.2(1), 2.3 ± 0.7(4), 2.7 ± 0(2), 4.1 ± 0.4(6), 0.9 ± 0.5(3), 2.4 ± 0.8(2), 4.8 ± 0.7(10), 3.0 ± 0.9(6), 1.2 ± 0.4(7).

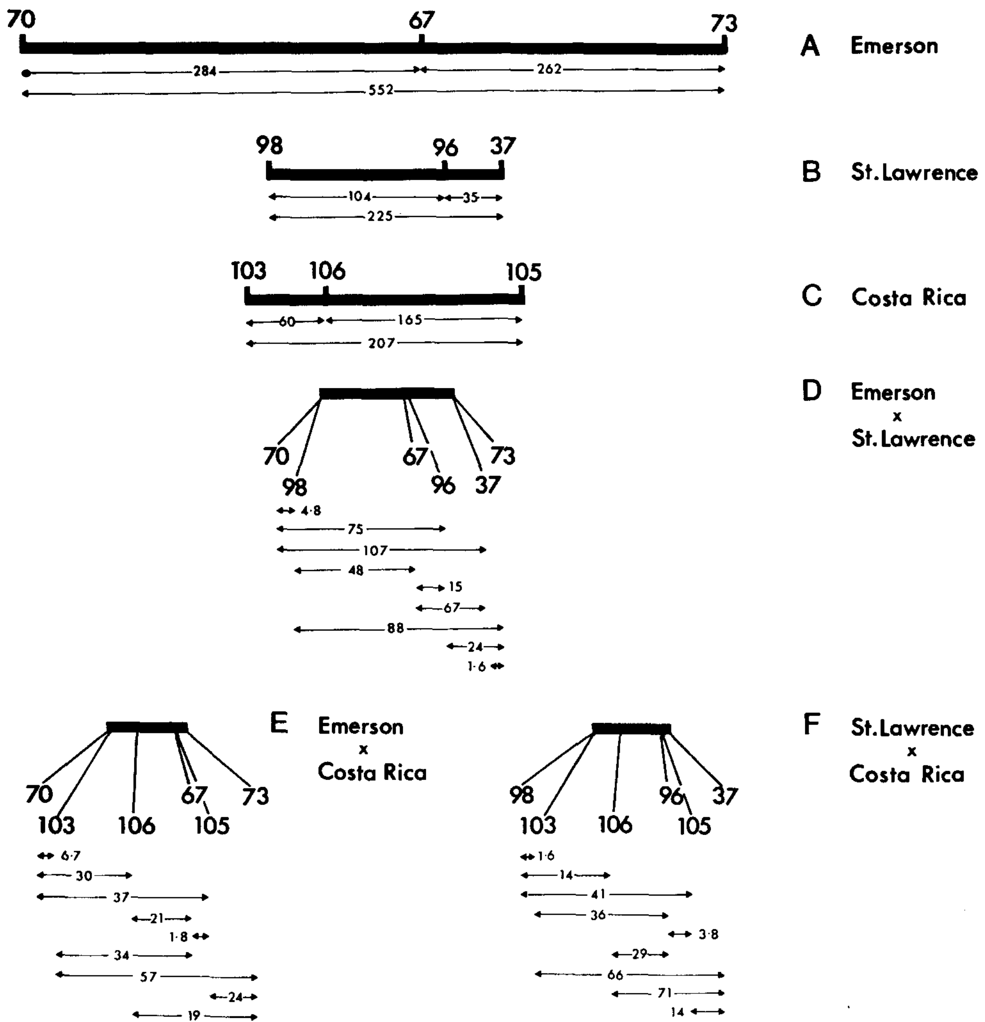


FIGURE 5.—Maps of the *nit-2* locus based upon crosses between mutants isolated in various wild types and homozygous *rec-1*. The crosses are between: (A) Emerson alleles, homozygous ss^E; (B) St. Lawrence alleles, homozygous ss^S; (C) Costa Rica alleles, homozygous ss^C; (D) Emerson and St. Lawrence alleles, heterozygous ss^E/ss^C; (F) St. Lawrence and Costa Rica alleles, heterozygous ss^S/ss^C.

The fiducial limits for the recombinational frequencies in 5A, 5B and 5D are calculable from the data in Table 3. For the remainder they are as follows (mean ± SD of mean or SE of estimate (number of crosses analyzed)): 5C: 60 ± 8(5), 165± 19(9), 207 ± 17(8); 5D: 6.7 ± 0.6(4), 30 ± 2(5), 37 ± 5(4), 21 ± 4(3), 1.8 ± 0.5(6), 34 ± 3(6), 57 ± 5(9), 24 ± 4(10), 19 ± 4(1); 5F: 1.6 ± 0.6(4), 14 ± 2(1), 41 ± 4(2), 36 ± 4(2), 3.8 ± 1.8(2), 29 ± 3(2), 66 ± 5(2), 71 ± 5(3), 14 ± 2(3).

TABLE 4

Recombination frequencies in crosses between nit-2 *alleles, induced in various wild types, and Emerson alleles*

nit-2 mutant			Prototroph frequency × 10^{-5} in crosses to Emerson alleles*		Recombination in the MN70 MN73 interval as a percentage of that expected†
Induced in wild type	Allele number	*rec-1* constitution of crosses	MN70	MN73	
Costa Rica	MN107	*rec-1*	44 ± 3	25 ± 5	13
251/12	MN111	+	33 ± 3	38 ± 5	60
Chilton	MN112	+	15 ± 2	9 ± 2	19
Liberia	MN115	*rec-1*	16 ± 2	68 ± 9	15
Beadle	MN116	+	35 ± 4	34 ± 3	58

* Prototroph frequencies were determined from a single cross, except for those for Chilton, each of which represents pooled data from two crosses. The MN70 stocks were 1971, 3988 and 2159; the MN73 stocks were 1199, 1373 and 2207.

† Σ recombination for the allele with each of MN70 and MN73 as a percentage of the recombination observed in crosses between MN70 and MN73 (see Figure 2).

DISCUSSION

Although like *cog* and *con*, *ss* genes affect recombination at or near their own location, the *ss* genes seem to be distinct. Both *con* and *cog* interact with *rec*. In the case of *cog* the same low frequency of recombination occurs in the presence of *rec*+ irrespective of the *cog* genotype; in the case of *con* an interaction between *rec* and *con* is indicated by the differential effect of *rec-3* on recombination at the *am-1* and *his-2* loci. However, *ss* does not interact with *rec*; it acts multiplicatively, changing the range of recombination frequencies, but not the relative magnitude of the high levels in crosses homozygous for *rec-1* to the low levels in crosses heterozygous for *rec-1/rec-1*+. The genes *ss* and *cog* are further distinguished by the dominance relationships between alleles. In the case of *cog*, one allele is dominant and gives high frequencies of recombination, while *ss* alleles yield characteristic frequencies of recombination in homozygous crosses and much lower frequencies in any heterozygous combination.

A number of hypotheses have been considered that could account for the suppression of recombination in crosses heterozygous for *ss*.

A trivial explanation for the suppression of *nit-2* recombination in crosses between Emerson, St. Lawrence and Costa Rica alleles is that each wild type differs from the others in the structural arrangement of this section of the chromosome in linkage group I. Such structural differences could arise from translocations or inversions in which at least one of the breakpoints is close to the *nit-2* locus. Heterozygosity for such rearrangements might reduce recombination by thwarting proper chromatid pairing or by eliminating certain recombinant products from the viable progeny due to their deficiency in essential genetic information.

Two types of evidence militate against the involvement of such gross chromosomal rearrangements: crosses in which the *nit-2* region of linkage group I is derived from different wild types do not exhibit the abnormally high frequencies

of ascospore abortion expected of crosses heterozygous for a chromosomal rearrangement, and recombination in the regions flanking the *nit-2* locus is not suppressed (Table 5). The effect of heterozygosity for *ss* is restricted to a region in the vicinity of the *nit-2* locus too small to be detected by measuring recombination between flanking markers. There is evidence of some variation in recombination in the regions flanking *nit-2* (Table 5); however, this is not correlated with variation in *ss* alleles and may be due to genes having small effects on recombination segregating in the crosses used to construct the stocks. There is good evidence for genes having effects of this magnitude on recombination in the *nit-2* locus (CATCHESIDE 1970, 1974).

An alternative explanation of the apparent shortfall in *nit-2*$^+$ recombinants in crosses among Emerson, St. Lawrence and Costa Rica alleles is that the coding sequences of the three wild type genes are not identical. If this were the case and if the differences were multiple, then recombination between *nit-2* alleles from the different wild types would generate novel coding sequences that might well confer a mutant phenotype. Thus, instead of generating *nit-2*$^+$ progeny, such events would yield *nit-2* products and remain cryptic. A prediction of this hypothesis is that crosses heterozygous for the *nit-2*$^+$ genes from two of the wild types should generate recombinant progeny with a *nit-2* mutant phenotype. To test this hypothesis, the following stocks were used: 3737, which is *A*; *cot-1*; *am-1 rec-1* and contains the *nit-2*$^+$ gene from the St. Lawrence wild type, and 9891, which is *a*; *cot-1*; *am-1 rec-1* and contains the *nit-2*$^+$ gene from the Emerson wild type. Altogether, 3018 random spores from a cross between 3737 and 9891 were picked on to alanine-supplemented medium; 2565 (85%) germinated and were tested for a *nit-2* phenotype. All were *nit-2*$^+$. An additional 1280 random colonies from ascospores germinated on alanine-supplemented medium were also tested for a *nit-2* phenotype. Again, all were *nit-2*$^+$. If the *nit-2*$^+$ genes are dissimilar in Emerson and St. Lawrence, a prediction of the frequency of *nit-2* progeny expected from this cross can be arrived at from the data in Figure 5. A pooled estimate for the length of the *nit-2* gene based on the frequency of recombination events generating prototrophs is 5.49×10^{-3} for Emerson alleles, 1.82×10^{-3} for St. Lawrence alleles and 1.04×10^{-3} when both Emerson and St. Lawrence alleles are involved. To account for the observed reduction in crosses heterozygous for Emerson and St. Lawrence *nit-2* genes, recombination events generating novel *nit-2* alleles of mutant phenotype would need to eliminate between 0.78×10^{-3} and 4.44×10^{-3} prototrophic recombinants. Since this reduction in the yield of prototrophs would detect only one of the two reciprocal classes of recombinant with a novel *nit-2* sequence, a cross of heterozygous *nit-2*$^+$ Emerson, *nit-2*$^+$ St. Lawrence would be expected to generate twice this frequency of progeny with *nit-2* mutant phenotypes; that is, between 1.56×10^{-3} and 8.88×10^{-3}. Since no *nit-2* recombinants were found in a total of 3845 progeny, the frequency of generation of such recombinants is less than 8.88×10^{-3} with a probability of $>> 0.999$, and less than 1.56×10^{-3} with a probability of 0.998. Therefore, it is unlikely that the reduced frequency of prototrophic recombinants

TABLE 5

Effect of heterozygosity for ss *on recombination in regions flanking the* nit-2 *locus*

Cross number	Stock number of *nit2*+ parent	Progeny: Parental *un-5*	Parental +	Recombinant: Region 1 *un-5*	Region 1 +	Region 2 *un-5*	Region 2 +	Region 1 & 2 *un-5*	Region 1 & 2 +	*ss* genotype	Percentage: Recombination (*un-5* — 1 — *nit-2* — 2 — *A*) 1	Recombination 2	Germination	White spores*
10205	9901	44	50	1	—	13	14	—	—	$ss^S \times ss^S$	0.8	22.1	95.3	1.3 ± 0.5
10206	9902	41	40	—	—	13	10	—	—	$ss^S \times ss^S$	0	22.1	81.3	< 0.4
10203	9892	39	51	1	3	17	11	—	—	$ss^S \times ss^E$	3.3	23.0	95.3	< 0.7
10204	9893	36	37	—	2	19	16	—	1	$ss^S \times ss^E$	2.7	32.4	86.7	0.7 ± 0.5
10208	9899	43	33	2	2	13	13	—	—	$ss^S \times ss^C$	3.6	23.4	86.7	0.7 ± 0.4
10209	9900	46	49	—	1	12	9	—	1	$ss^S \times ss^C$	1.7	18.6	92.2	1.8 ± 0.6

Crosses were made between stock 5557 (*un-5* ss^S *nit-2*(nr37) *A*; *cot-1*; *am-1 rec-1*) and stocks of genotype *a*; *cot-1*; *am-1 rec-1*, in which the *nit-2*+ gene was derived from either Emerson, St. Lawrence or Costa Rica wild types. The distribution of progeny genotypes is similar in each cross, $\chi^2_{30} = 24.2$.

* ± Standard error of the estimate or, where no white spores were observed in the sample, the 95% upper confidence limit.

in crosses heterozygous for the *nit-2* genes of Emerson and St. Lawrence is due to dissimilar coding sequences in the $nit\text{-}2^+$ gene of these two wild types.

A third possible explanation for the low frequency of prototrophic recombinants in crosses between *nit-2* alleles induced in Emerson, St. Lawrence and Costa Rica is that each of these wild types carries different alleles of a gene (*ss*) that determines a step in recombination events occurring in the vicinity of the *nit-2* locus. Since heterozygosity for *ss* is associated with a reduction in recombination, the plausible roles that *ss* might play in recombination are limited. A straightforward explanation of the low frequency in heterozygotes is to propose that the *ss* genes determine the pairing closeness of homologous DNA duplexes in the vicinity of the *nit-2* gene. Thus, the *ss* genotype would determine the frequency of formation of intimately paired duplexes, a necessary prelude to recombination and the formation of the substrate upon which enzymes promoting recombination can act. Pairing would be expected to be poor in crosses heterozygous for $ss^S \times ss^E$, $ss^E \times ss^C$ and $ss^S \times ss^C$ and recombination frequencies concomitantly low. In crosses homozygous for ss^S, ss^E or ss^C, pairing would be expected to be effective and recombination frequencies high.

It is possible that *ss* influences an event later in recombination rather than or as well as DNA duplex formation. Following synapsis, recombination is thought to proceed as follows: a cut in one strand of the DNA at the cog^+ site of one duplex catalyzed by an endonuclease, single-strand displacement by DNA polymerase, D loop formation and the generation of a single-strand bridge to form an asymmetric heteroduplex, isomerization to generate a two-strand bridge and a symmetric heteroduplex, repair of mismatched bases and scission of the cross-strand bridges (MESELSON and RADDING 1975; D. G. CATCHESIDE and ANGEL 1974). Although modulation of DNA pairing is a simple explanation of the low recombination frequencies observed in crosses heterozygous for *ss*, other explanations are possible. For example, asymmetry in the paired duplexes due to heterozygosity for *ss* alleles could interfere with D loop formation, or with the migration of single- or double-strand cross connections. A need for close sequence symmetry in any of the other steps of recombination is not apparent, and it seems unlikely that modulation of one of these steps could explain the low frequency of $nit\text{-}2^+$ recombinants in crosses heterozygous for *ss*.

In addition to the low frequency of recombinants in heterozygous crosses, two properties of *ss* are consistent with its playing a role in determining the frequency of close synapsis of DNA in the *nit-2* region. First, if the role of *ss* genes is to determine the efficiency of local pairing, there is no compelling reason why different alleles should be equally effective in establishing pairing. Indeed, for genes whose role is to modulate recombination, it would be reasonable to expect different recombination frequencies to be associated with specific alleles. Consistent with this expectation, crosses homozygous for ss^E, ss^C or ss^S do not yield identical frequencies of $nit\text{-}2^+$ recombinants; the map of the *nit-2* locus constructed from crosses homozygous for ss^E is longer than that constructed from crosses homozygous for ss^S or ss^C (Figures 4 and 5), implying that ss^E catalyzes more effective or more frequent pairing than does either ss^S or ss^C. This finding

that homozygosity for different *ss* alleles is not always associated with the same recombination frequency is not a prediction of the alternative hypothesis that *ss* heterozygosity interferes with D loop formation or bridge migration, and makes these explanations less probable. Second, if the function of *ss* is to establish localized close pairing of DNA as a precondition for the initiation of recombination, it is expected that the effect of genes modulating a step in the recombination event itself should not be influenced by *ss*. The multiplicative interaction between the effects of $rec\text{-}1^+$ and the *ss* alleles ss^E and ss^S (Figures 4A, B, D and 5A, B, D) is consistent with this expectation. $rec\text{-}1^+$-mediated control of recombination is envisaged as acting on paired DNA duplexes, preventing the initiation of recombination events at a cog^+ site close to *nit-2* (CATCHESIDE 1974). Variation in the frequency of pairing of the *nit-2* region should act as a multiplier of the $rec\text{-}1^+$ modulation of recombination between *nit-2* alleles.

In crosses heterozygous for ss^S/ss^C and ss^E/ss^C (Figures 4E, F and 5E, F), the effect of $rec\text{-}1^+$ is to reduce recombination 11.4- and 11.5-fold, respectively. Apparently, this is somewhat greater than in crosses heterozygous ss^E/ss^S or homozygous for ss^E, ss^S or ss^C, where the effect of $rec\text{-}1^+$ is to reduce recombination 5.7-, 4.6-, 4.1- and 5.7-fold, respectively. This deviation from a precise multiplicative relationship between the *ss* and $rec\text{-}1^+$ effects could reflect a minor interaction of the $rec\text{-}1^+$ gene product with *ss*; for example, that the $rec\text{-}1^+$ product binds to DNA prior to close synapsis and in consequence reduces the already poor chance of ss^C initiating pairing with ss^E or ss^S. However, this deviation from a precise multiplicative relationship could also be accounted for by the genes of minor effect already mentioned. Thus, there is no strong evidence that the *ss* and $rec\text{-}1^+$ effects are other than independent.

The precise location of *ss* with respect to the *nit-2* locus is of interest. The genetic data place it less than twice the length of the *nit-2* coding sequence from the *nit-2* locus and do not exclude the possibility that it lies within the locus as an intervening sequence. Allelic variation in intervening sequences has been found in the chicken ovalbumin gene (LAI *et al.* 1979). However, intervening sequences have not yet been reported for nuclear genes in Neurospora, although they are known to occur in the 24S rRNA gene in Neurospora mitochondria (HAHN *et al.* 1979).

The high incidence of variation in *ss* genes among Neurospora strains raises the possibility that they are transposable elements. Transposable elements have not been reported in Neurospora but are known to occur in a number of eukaryotes, including maize (MCCLINTOCK 1956), Drosophila (POTTER *et al.* 1979) and yeast (CAMERON, LOH and DAVIS 1979). Determination of the nature and location of the *ss* sequences will probably require physical analysis of DNA from the *nit-2* regions of the appropriate Neurospora strains.

Although nonspecific interference with a step in recombination subsequent to synapsis cannot be excluded as the basis for the *ss* effect, a plausible and possible function for *ss* genes would be as sites determining pairing closeness in the vicinity of the *nit-2* gene, this close pairing being necessary for the successful initiation of recombination events from cog^+ loci. Sites with this function might

be widely distributed through the genome and supplement the *rec* system in controlling recombination in specific chromosomal regions. Jointly, *ss* and *rec-1*+ control recombination between *nit-2* alleles over a 100-fold range. Natural variation in the postulated *cog* and *con* sites close to *nit-2*, as is known to occur in other sections of the Neurospora genome (ANGEL, AUSTIN and D. G. CATCHESIDE 1970; D. G. CATCHESIDE 1975), might act as further multipliers of this range, extending it as much as 10^3- to 10^4-fold, offering a means of stabilizing particular gene combinations by ensuring tight linkage in meiosis, yet preserving the possibility of high-frequency recombination in future generatons.

Variation in recombination attributable to local heterozygosity for genetic background is not always associated with suppression of recombination. DE SERRES (1971) reported that in crosses between *ad-3A* and *ad-3B* in Neurospora, recombination frequency was higher when the mutants originated in different strains than when the mutants were derived from the same strain. This is the converse of what might be expected from the present study, and is not readily explicable in terms of variation in the known type of unlinked gene (*rec*) that can influence recombination in Neurospora. Nevertheless, genes with properties similar to *ss* have been reported to modulate recombination in spore pigmentation genes of *Ascobolus immersus* (EMERSON and YU-SUN 1967; GIRARD and ROSSIGNOL 1974) and the *adh1* locus of maize (FREELING 1978). Thus, it is possible that genes determining synapsis are widely distributed in eukaryotes. In diploid organisms, in which pairing of genes may be important for gene regulation (JACK and JUDD 1979), *ss* genes could have a role in somatic cells.

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